Nutrition in the Prevention and Treatment of Disease

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Fourth Edition

Edited by

Ann M. Coulston, MS, FAND
Santa Fe, NM, United States

Carol J. Boushey, PHD, MPD, RD
University of Hawaii Cancer Center, Honolulu, HI, United States

Mario G. Ferruzzi, PHD
North Carolina State University, Kannapolis, NC, United States

Linda M. Delahanty, MS, RD, LDN
Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States

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List of Contributors

Tim Ackland, The University of Western Australia, Crawley, WA, Australia

Leiddy Z. Alvarado, Albany Molecular Research Inc., Rensselaer, NY, United States

Cheryl A.M. Anderson, University of California at San Diego, La Jolla, CA, United States

Caroline M. Apovian, Boston University School of Medicine, Boston, MA, United States

Merlin W. Ariefdjohan, Purdue University, West Lafayette, IN, United States

Marion Taylor Baer, University of California at Los Angeles, Los Angeles, CA, United States

Jennifer L. Barnes, Illinois State University, Normal, IL, United States

Bryan C. Batch, Duke University Medical Center, Durham, NC, United States

Sinead N. Bhriain, Trinity College, University of Dublin, and Dublin Institute of Technology, Dublin, Ireland

Courtney K. Blomme, University of Wisconsin-Madison, Madison, WI, United States

George A. Bray, Pennington Biomedical Research Center/LSU, Baton Rouge, LA, United States

Onikia N. Brown-Esters, Purdue University, West Lafayette, IN, United States

Lora E. Burke, University of Pittsburgh School of Nursing, Pittsburgh, PA, United States

Carol J. Boushey, University of Hawaii Cancer Center, Honolulu, HI, United States

Lisa Cadmus-Bertram, University of Wisconsin-Madison, Madison, WI, United States

Mona S. Calvo, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Laurel, MD, United States

Sara C. Campbell, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States

Lyanne H. Chin, University of Wisconsin, Madison, WI, United States

Rebecca B. Costello, National Institutes of Health, Bethesda, MD, United States

Melissa M. Crane, HealthPartners Institute, Minneapolis, MN, United States; University of Minnesota, Minneapolis, MN, United States

Amanda J. Cross, Imperial College, London, United Kingdom

Pamela Cureton, MassGeneral Hospital *for* Children, Boston, MA, United States; Harvard Medical School, Boston, MA, United States

Aurélie de Rus Jacquet, Purdue University, West Lafayette, IN, United States; Howard Hughes Medical Institute, Seattle, WA, United States

Linda M. Delahanty, Harvard Medical School, Boston, MA, United States; Massachusetts General Hospital Diabetes Center, Boston, MA, United States

James P. DeLany, University of Pittsburgh, Pittsburgh, PA, United States

Wendy Demark-Wahnefried, University of Alabama at Birmingham, Birmingham, AL, United States

Abby Dilk, Purdue University, West Lafayette, IN, United States

V. Drapeau, Laval University, Quebec, QC, Canada

Maria Duarte-Gardea, The University of Texas at El Paso, El Paso, TX, United States

Johanna T. Dwyer, National Institutes of Health, Bethesda, MD, United States

Alessio Fasano, MassGeneral Hospital *for* Children, Boston, MA, United States; Harvard Medical School, Boston, MA, United States

Mario G. Ferruzzi, North Carolina State University, Kannapolis, NC, United States

Janis S. Fisler, University of California, Davis, CA, United States

Jo L. Freudenheim, University at Buffalo, The State University of New York, Buffalo, NY, United States

Daniel D. Gallaher, University of Minnesota, St. Paul, MN, United States

Karen Glanz, University of Pennsylvania, Philadelphia, PA, United States

Katheryn M. Goodrich, Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Emily Gower, University at Buffalo, The State University of New York, Buffalo, NY, United States

Martha Guevara-Cruz, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico City, Mexico

Earl Harrison, Ohio State University, Columbus, OH, United States

Yoriko Heianza, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States

Holly Herrington, Center for Lifestyle Medicine, Northwestern Memorial Group, Chicago, IL, United States

Steve Hertzler, Abbott Laboratories, Ross Products Division, Columbus, OH, United States

Kathleen M. Hill Gallant, Purdue University, West Lafayette, IN, United States

Patricia A. Hume, Auckland University of Technology, Auckland, New Zealand

Karry A. Jackson, Purdue University, West Lafayette, IN, United States

Wei Jia, University of Hawaii Cancer Center, Honolulu, HI, United States; Shanghai Jiao Tong University School of Medicine, Shanghai, China

Rachel K. Johnson, The University of Vermont, Burlington, VT, United States

Deborah A. Kerr, Curtin University, Perth, WA, Australia

Kee-Hong Kim, Purdue University, West Lafayette, IN, United States

Laurence N. Kolonel, University of Hawaii, Honolulu, HI, United States

Penny Kris-Etherton, Pennsylvania State University, State College, PA, United States

Robert F. Kushner, Northwestern University Feinberg School of Medicine, Chicago, IL, United States; Center for Lifestyle Medicine, Northwestern Medicine, Chicago, IL, United States

HuiChuan J. Lai, University of Wisconsin, Madison, WI, United States

Johanna W. Lampe, Fred Hutchinson Cancer Research Center, Seattle, WA, United States

Thomas P. Lawler, University of Wisconsin-Madison, Madison, WI, United States

Ki Won Lee, Seoul National University, Seoul, Republic of Korea

Maureen M. Leonard, MassGeneral Hospital *for* Children, Boston, MA, United States; Harvard Medical School, Boston, MA, United States

Pao-Hwa Lin, Duke University Medical Center, Durham, NC, United States

Juliet Mancino, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

Robert Marcus, Stanford University, Stanford, CA, United States

Julie A. Mares, University of Wisconsin-Madison, Madison, WI, United States

Joyce Merkel, National Institutes of Health, Bethesda, MD, United States

Amy E. Millen, University at Buffalo, The State University of New York, Buffalo, NY, United States

Barbara Millen, Boston University School of Medicine, Boston, MA, United States; Millennium Prevention, Inc, Westwood, MA, United States

G.A. Nagana Gowda, University of Washington, Seattle, WA, United States

Andrew P. Neilson, Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Mihai D. Niculescu, Advanced Nutrigenomics LLC, Concord, NC, United States

Beth Ogata, University of Washington, Seattle, WA, United States

Jose M. Ordovas, Tufts University, Boston, MA, United States; Centro Nacional Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Kenya D. Palmer, Boston University School of Medicine, Boston, MA, United States

Song-Yi Park, University of Hawaii, Honolulu, HI, United States

Ruth E. Patterson, University of California, San Diego, La Jolla, CA, United States

Toni I. Pollin, University of Maryland School of Medicine, Baltimore, MD, United States

S. Pomerleau, Laval University, Quebec, QC, Canada

Kathleen J. Porter, University of Virginia, Charlottesville, VA, United States

V. Provencher, Laval University, Quebec, QC, Canada

Lu Qi, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States; Harvard T.H. Chan School of Public Health, Boston, MA, United States

Daniel Raftery, University of Washington, Seattle, WA, United States; Fred Hutchinson Cancer Research Center, Seattle, WA, United States

Cynthia Rajani, University of Hawaii Cancer Center, Honolulu, HI, United States

Chesney Richter, The University of Arizona, Tucson, AZ, United States

Kim Robien, University of Minnesota, Minneapolis, MN, United States

Jean-Christophe Rochet, Purdue University, West Lafayette, IN, United States

Cheryl L. Rock, University of California at San Diego, La Jolla, CA, United States

Dennis A. Savaiano, Purdue University, West Lafayette, IN, United States

TusaRebecca E. Schap, Alexandria, VA, United States

Elisabeth M. Seburg, HealthPartners Institute, Minneapolis, MN, United States

Harold E. Seifried, National Cancer Institute, Rockville, MD, United States

Rebecca M. Seifried, Walter Reed National Military Medical Center, Bethesda, MD, United States

Nancy E. Sherwood, HealthPartners Institute, Minneapolis, MN, United States; University of Minnesota, Minneapolis, MN, United States

Ann Skulas-Ray, The University of Arizona, Tucson, AZ, United States

Linda G. Snetselaar, University of Iowa, Iowa City, IA, United States

Fabrizis L. Suarez, Abbott Laboratories, Ross Products Division, Columbus, OH, United States

Amy F. Subar, National Cancer Institute, Bethesda, MD, United States

Laura P. Svetkey, Duke University Medical Center, Durham, NC, United States

Mitali A. Tambe, Purdue University, West Lafayette, IN, United States; Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, United States

Kelly A. Tappenden, University of Illinois at Urbana-Champaign, Urbana, IL, United States

Alyce M. Thomas, St. Joseph's Regional Medical Center, Paterson, NJ, United States

Frances E. Thompson, National Cancer Institute, Bethesda, MD, United States

Sabrina P. Trudo, University of Arkansas, Fayetteville, AR, United States

Crystal C. Tyson, Duke University Medical Center, Durham, NC, United States

Hassan Vatanparast, University of Saskatchewan, Saskatoon, SK, Canada

Adriana Villaseñor, University of California, San Diego, La Jolla, CA, United States

Craig H. Warden, University of California, Davis, CA, United States

Connie M. Weaver, Purdue University, West Lafayette, IN, United States

Susan J. Whiting, University of Saskatchewan, Saskatoon, SK, Canada

Hope Wills, University of Southern California, Los Angeles, CA, United States

Paul J. Wisniewski, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States

Judith Wylie-Rosett, Albert Einstein College of Medicine, Bronx, NY, United States

Zhumin Zhang, University of Wisconsin, Madison, WI, United States

Yaguang Zheng, Boston College Connell School of Nursing, Chestnut Hill, MA, United States

Jamie M. Zoellner, University of Virginia, Charlottesville, VA, United States

About the Editors

Ann M. Coulston
Ann Coulston is retired from the practice of nutrition and dietetics. She has a more than 20-year history of clinical research at Stanford University Medical Center where her research centered on carbohydrate and lipid metabolism, the nutritional management of diabetes, and insulin resistance. She has provided nutrition consultation to the food and healthcare industry, public relations firms, and Internet companies. She is Past-President of the Academy of Nutrition and Dietetics (formerly the American Dietetic Association) and has been recognized by the Academy of Nutrition and Dietetics Foundation for excellence in the practice of clinical nutrition and the practice of research.

Carol J. Boushey
Carol Boushey is an Associate Research Professor in the Epidemiology Program at the University of Hawaii Cancer Center and an Adjunct Professor in Nutrition Science at Purdue University. Her research focuses on dietary assessment and examining the relationship of dietary intakes or dietary patterns as an exposure for health or risk for disease. Her research has appeared in book chapters and journals, such as the *Journal of the Academy of Nutrition and Dietetics (JAND)*, *Pediatrics, the Journal of Nutrition, the American Journal of Clinical Nutrition*, and *JAMA*. She received a BSc from the University of Washington, a Masters of Public Health from the University of Hawaii at Manoa, and a PhD from the University of Washington through the interdisciplinary nutrition program and the epidemiology program.

Mario G. Ferruzzi
Mario Ferruzzi is a Professor in the Plants for Human Health Institute and the Department of Food, Bioprocessing and Nutrition Science at North Carolina State University. He received his BS in Chemistry from Duke University and PhD in Food Science and Nutrition from the Ohio State University. Previously, Dr. Ferruzzi served as a Professor in the Department of Food Science at Purdue University and previously as a Research Scientist with Nestlé Research & Development. His research focuses on understanding the impact of food formulation and processing on bioavailability, metabolism and biological activity of plant bioactives and micronutrients. He is a Member of the Institute of Food Technologist (IFT), the American Society for Nutrition (ASN), the American Chemical Society (ACS), and the Royal Society of Chemistry (RSC). He currently serves on the editorial board for Critical Reviews in Food Science and Nutrition, Nutrition Research, Nutrition Today and as an Associate Editor for the journal *Food & Function*.

Linda M. Delahanty
Linda Delahanty is Chief Dietitian and Director of Nutrition and Behavioral Research at Massachusetts General Hospital's Diabetes Center and Assistant Professor of Medicine at Harvard Medical School. She is a nationally recognized authority on nutrition and lifestyle interventions for the treatment of diabetes and obesity. She was a recipient of the National Award for Excellence in the Practice of Dietetics Research and the Medallion Award for lifetime achievement in the dietetics profession from the Academy of Nutrition and Dietetics and received the American Diabetes Association's Outstanding Educator in Diabetes Award in 2015. She received her BS in Nutrition and Food Science from the University of Massachusetts Amherst and her MS in Clinical Nutrition from Boston University. Her research focuses on the design of nutrition and lifestyle interventions and examining their impact on diabetes, obesity, hyperlipidemia, breast cancer, quality-of-life and genetics outcomes in both clinical and comparative effectiveness trials.

Preface

Our purpose in creating this text is to provide a compilation of current knowledge in clinical nutrition and an overview of the rationale and science base of its application to practice in the prevention and treatment of disease. The first section addresses the basic principles and concepts that are central to clinical nutrition research methodology. Because nutrition information is gathered from a variety of study designs, research methodology, epidemiology, and intervention studies are reviewed, coupled with data analysis, intervention techniques, and application of behavioral principles to nutrition intervention. In this edition, we have added two new chapters on topics that have come to be used in nutrition research. Metabolomics is discussed in Chapter 5 followed by a technique starting to be used in clinical research, Translational Research in Chapter 6. Throughout these chapters, new areas of study are discussed with the perspective that the application of the scientific method is by definition an evolutionary process. Specific examples, drawn from recently published reports, bring the principles to life.

The second section covers areas of study that contribute to knowledge in clinical nutrition, including disease-relevant biochemistry, metabolism, dietary factors within tissues and cells, and attitudes about food and the eating patterns and behaviors of targeted individuals or groups. This section presents a rich array of topics that cover areas of general interest and nutrition guidelines.

We are continuing with topics on dietary bioactive compounds for health, which explores bioactive components present in edible plants of particular interest for the prevention of disease. Their widespread use has the potential to impact human health on the population level. Uses of these compounds are explored in cognition, eye disease, and obesity. Also, physiological factors that enhance digestion, absorption, and metabolism bring a greater understanding of bioactives to overall health.

Clinical nutrition is the aspect of nutrition science that is related to the development, progression, or management of disease, as differentiated from the issues of normal requirements, cellular functions, and activities. Interventions range from efforts to maintain health during short-term illness to optimization of health status in individuals at risk for or diagnosed with chronic diseases and to major nutritional and dietary modifications as specific or adjuvant treatments for disease. The first condition addressed is the ever-growing concern with overweight and obesity. As with many of the following disease groups, this grouping begins with a chapter on the genetics of human obesity and moves on to issues related to treatment, the role of physical activity, nutrient-related considerations, childhood and adolescent issues, environmental cues controlling energy intake, and surgical therapies.

Cardiovascular disease, also a condition closely related to nutrition, is summarized in three chapters that examine genetic considerations, lipid disorders, and hypertension. Closely related to obesity and cardiovascular disease is diabetes mellitus. It is interesting how many of the clinical nutrition areas interrelate: Obesity is a risk factor for cardiovascular disease and diabetes, whereas diabetes is an independent risk factor for cardiovascular disease. New to the section on diabetes is a chapter on Genetics and Diabetes. Dietary intake or nutritional status may be altered as a result of disease or by the treatment modalities that are used, such as surgical treatments or medical management strategies, including prescription medications. The altered needs must be met by dietary or nutrition interventions in order to prevent malnutrition and the associated consequences that contribute to morbidity and mortality.

Nutrition intervention can be a critical component of disease prevention, an important aspect of disease management, or the primary treatment for disease. This is exemplified by the chapters dealing with cancer, beginning again with a discussion of the genetic components, followed by a discussion of malignancies that have connections to nutrition and specific nutrients. Gastrointestinal diseases, especially the newer knowledge about diet and microflora of the gastrointestinal tract, demonstrate the importance of food choices in disease prevention, treatment, and management. New to the Gastrointestinal section is a chapter on the Microbiome, a burgeoning area many believe will offer new and helpful treatment information. The bone health chapters cover three important topics linked by the nutrients calcium and

vitamin D and tell an important story of the value of early nutrition on health in later years.

Generating and analyzing data that summarize dietary intake and its association with disease are valuable tasks in treating disease and developing disease-prevention strategies. Well-founded medical nutrition therapies can minimize disease development and related complications. Providing scientifically sound, creative, and effective nutrition interventions is challenging and rewarding. We plan to update our knowledge and its application through future editions of this text.

Ann M. Coulston

Carol J. Boushey

Mario G. Ferruzzi

Linda M. Delahanty

Chapter 1

Dietary Assessment Methodology

Frances E. Thompson and Amy F. Subar
National Cancer Institute, Bethesda, MD, United States

I INTRODUCTION

This chapter is a revision of the similarly named chapter in the earlier editions [1−3] of this book, which itself was based on the "Dietary Assessment Resource Manual" [4] by Frances E. Thompson and Tim Byers, adapted with permission from the *Journal of Nutrition*. Dietary assessment encompasses food supply and production at the national level, food purchases at the household level, and food consumption at the individual level. This review focuses only on individual-level food intake. It is intended to serve as a resource for those who wish to assess diet in a research study, for example, to describe the intakes of a population, using individual measurements for group-level analysis. This chapter does not address clinical assessment of individuals for individual counseling. The first section reviews major dietary assessment methods, their advantages and disadvantages, and validity. The next sections describe which dietary assessment methods are most appropriate for different types of studies and for various types of populations. Finally, specific issues that relate to all methods are discussed.

II DIETARY ASSESSMENT METHODS

A Dietary Records

In the dietary record approach, the respondent records the foods and beverages and the amounts of each consumed over one or more days. Ideally, the recording is done at the time of the eating occasion in order to avoid reliance on memory. The amounts consumed may be measured, using a scale or household measures (e.g., cups or tablespoons), or estimated using models, pictures, or no aid. If multiple days are recorded, they are usually consecutive, and no more than 7 days are included. Recording periods of more than 4 consecutive days are usually unsatisfactory, as reported intakes decrease [5] due to respondent fatigue, and individuals who do comply may differ systematically from those who do not. Because the foods and amounts consumed on consecutive days of reporting may be related (e.g., leftovers and eating more one day and less the next day), it may be advantageous to collect nonconsecutive single-day records in order to increase representativeness of the individual's diet.

To complete a dietary record, each respondent must be trained in the level of detail required to adequately describe the foods and amounts consumed, including the name of the food (brand name, if possible), preparation methods, recipes for food mixtures, and portion sizes. In some studies, this is enhanced if the investigator contacts the respondent and reviews the report after 1 day of recording. At the end of the recording period, a trained interviewer should review the records with the respondent to clarify entries and to probe for forgotten foods [6]. Dietary records also can be recorded by someone other than the subject, such as parents reporting for their children.

The dietary record method has the potential for providing quantitatively accurate information on food consumed during the recording period [7]. By recording foods as they are consumed, the problem of omission may be lessened and the foods more fully described. Furthermore, reporting amounts of food as they are consumed should provide more accurate portion size information than if the respondents were recalling portion sizes of foods previously eaten.

Although intake data using dietary records are typically collected in an open-ended form, close-ended forms have also been developed [8−10]. These forms consist of listings of food groups; the respondent indicates whether that food group has been consumed. In format, these "checklist" forms resemble food frequency questionnaires

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(FFQs) (see Section II.C). Unlike FFQs, which generally query about intake over a specified time period such as the past year or month, checklists are intended to be filled out concurrently with actual intake or at the end of a day for that day's intake. A checklist can be developed to assess particular "core foods" that contribute substantially to intakes of some nutrients [11], and it also has been used to track food contaminants [12]. Portion size can also be asked, either in an open-ended manner or in categories.

A potential disadvantage of the dietary record method is that it is subject to bias both in the selection of the sample and in the sample's completion of the number of days recorded. Dietary record keeping requires that respondents or respondent proxies be both motivated and literate (except for photograph-based methods), which can potentially limit the method's use in some population groups (e.g., low literacy, recent immigrants, children, and some elderly). The requirements for cooperation in keeping records can limit who will respond, compromising the generalizability of the findings from the dietary records to the broader population from which the study sample was drawn. Research indicates that incomplete records increase significantly as more days of records are kept, and the validity of the collected information decreases in the later days of a 7-day recording period, in contrast to information collected in the earlier days [5]. Part of this decrease may occur because many respondents develop the practice of filling out the record retrospectively rather than concurrently. When respondents record only once per day, the record method becomes similar to the 24-hour dietary recall in terms of relying on memory rather than concurrent recording.

An important disadvantage of this method is that recording foods as they are being eaten can affect both the types of food chosen and the quantities consumed [13–15]. The knowledge that foods and amounts must be recorded and the demanding task of doing it may alter the dietary behaviors the tool is intended to measure [16], creating "reactivity bias." This effect is a weakness when the aim is to measure typical dietary behaviors. However, when the aim is to enhance awareness of dietary behaviors and change them, as in some intervention studies, this effect can be seen as an advantage [17]. Recording, by itself, is an effective weight loss technique [18,19]. Recent interest in "real-time" assessment has led to the development of numerous mobile "apps" for self-monitoring that enable concurrent recording and immediate, automated feedback. This approach generally has been found to improve self-monitoring and adherence to dietary goals compared with traditional paper-and-pencil dietary records [20,21].

A third disadvantage is that unless dietary records are collected electronically, the data can be burdensome to code and can lead to high personnel costs. Dietary assessment software that allows for easier data entry using common spellings of foods can save considerable time in data coding. Even with high-quality data entry, maintaining overall quality control for dietary records can be difficult because information often is not recorded consistently among different respondents, nor is the information coded consistently among different coders. This highlights the need for training of both the respondents and the coders.

Several approaches using a variety of technological advances have been used to allow easier data capture and less respondent burden; some may be particularly beneficial among low-literacy groups. For example, a computer-administered instrument allows the respondent to select the food consumed and the appropriate portion size from photographs on a screen [22,23]; this can be done using touch-screen technology [24]. The proliferation of mobile devices with cameras allows simultaneous photographic records of the foods selected [25]. However, for this approach to be quantifiable, before and after pictures of a consumption event and training of the participant in how to consistently take pictures using a standard reference object are required. Wearable cameras which can continuously take pictures or videos have been developed [26,27], lessening the burden on the respondent and potentially allaying some reactivity (i.e., changes in the respondent's behavior that are caused by the instrument). These methods have great potential to improve portion size accuracy.

Automated processing of the image information for these methods is not yet fully developed. The images that illustrate the beginning of the consumption event and its completion must be selected, the food has to be identified [28], and the mathematical properties of the food image need to be quantified [29] in order to develop an accurate estimate of the food's volume. However, if these problems can be solved, the foods can be linked to appropriate databases (see Section V.E), dramatically reducing the burden of coding [30]. In the meantime, the images could be identified manually by staff or the respondent in an accompanying application, and later coded.

Respondent burden and reactivity bias may be less pronounced for the "checklist" [31], because checking off a food item is easier than recording a complete description of the food [32], and the costs of data processing can be minimal, for example, paper forms that are machine scannable, or electronic forms on a computer or mobile device. Checklists are often developed to assess particular foods that contribute substantially to intakes of some nutrients. As the comprehensiveness of the nutrients to be assessed increases, the length of the form also increases, and it becomes more burdensome to complete at each eating occasion and may increase reactivity. Nonetheless, precoded food diaries to assess diet have been developed,

evaluated, and used: the precoded food diary used in the 2005−08 Danish National Survey of Diet and Physical Activity contained about 400 items and portion size choices [33]; a precoded food diary used in Norway contained 277 items [34]. However, checklists are limited in their ability to assess the diet, because of lack of details on the particular food consumed, food preparation, portion sizes, and other relevant information.

Food records have been evaluated most frequently through comparison to another instrument, often 24-hour recalls. However, no self-report instrument is without reporting error, and thus relative validation is not necessarily useful. Instead, when possible, validation studies should consider using "recovery" biomarkers that are unbiased reference instruments. Only a few are currently available. These are total energy expenditure from doubly labeled water for energy [35], and protein (nitrogen) [36], potassium [37], and sodium based on 24-hour urine collections [38]. Many studies in selected small samples of adults indicate that reported energy and protein intakes on dietary records are underestimated in the range of 4−37% compared to energy expenditure as measured by doubly labeled water and protein intake as measured by urinary nitrogen [18,39−53]. In the largest doubly labeled water study using food records, with about 450 postmenopausal women in the Women's Health Initiative, energy and protein intakes reported on food records were underestimated by about 20% and 4%, respectively, and protein density (kcal of protein as a percentage of total kcal) was overestimated by about 17% [54]. Underreporting on dietary records is probably a result of the combined effects of incomplete and inaccurate recording and the impact of the recording process on dietary choices leading to undereating, and thus not typical of usual intake [18,48,55,56]. The highest levels of underreporting on dietary records have been found among individuals with greater body mass index (BMI) [41,43,44,54,57,58], particularly women [41,43,44,52,59−61]. This effect, however, may be due, in part, to the fact that overweight individuals are more likely to be dieting on any given individual day [62]. These relationships between underreporting and BMI and sex have also been found among elderly individuals [63]. Other research shows that demographic or psychological indices such as education, employment, social desirability, body image, or dietary restraint also may be important factors related to underreporting on diet records [41,48,60,61,64−67]. A few studies suggest that energy underreporters compared to others have reported intakes that are lower in absolute intake of most nutrients [58], higher in percentage of energy from protein [58,61], and lower in percentage of energy as carbohydrate [58,61,68,69] and in percentage of energy from fat [69]. Correspondingly, energy underreporters may report lower intakes of desserts, sweet baked goods, butter, and alcoholic beverages [58,69], but more grains, meats, salads, and vegetables [58]. Some research has examined the performance of food checklists relative to accelerometry [70] or, more commonly, complete dietary records [8,9,32], 24-hour dietary recalls [11], dietary history [71], and biological markers [71]. An evaluation study of the 7-day precoded food diary used in the Danish National Survey of Dietary Habits and Physical Activity 2000−02 reported that energy intake was underestimated by 12% compared to accelerometer [70].

Some approaches have been suggested to overcome underreporting in the dietary record. These include enhanced training of respondents and incorporating psychosocial questions known to be related to underreporting in order to control for the effect of underreporting [56]. Another approach is to calibrate dietary records to doubly labeled water or urinary nitrogen, biological indicators of energy expenditure and protein intake, respectively, including covariates of sex, weight, and height, to more accurately predict individuals' energy and protein intake [72]. This approach was applied to a subcohort of the Women's Health Initiative. Calibration equations that included BMI, age, and ethnicity explained much more of the variation in the energy and protein biomarkers than did calibration without the covariates, for example, 45% versus 8% for energy [54]. Further research is needed to test this approach in other populations and to develop and test other modeling approaches.

B 24-Hour Dietary Recall

In the 24-hour dietary recall, the respondent is asked to remember and report all the foods and beverages consumed in the preceding 24 hours or on the preceding day. The recall typically is conducted by interview, in person or by telephone [73,74], either computer-assisted [75] or using a paper-and-pencil form, although self-administered computer administration is becoming more prevalent [76−80]. When interviewer-administered, well-trained interviewers are crucial because much of the dietary information is collected by asking probing questions. Interviewers should be knowledgeable about foods available in the marketplace and about preparation practices, including prevalent regional or ethnic foods.

The interview is often structured, usually with specific probes, to help the respondent remember all foods consumed throughout the day. An early study found that respondents with interviewer probing reported 25% higher dietary intakes than did respondents without interviewer probing [81]. Probing is especially useful in collecting necessary details, such as how foods were prepared. It is also useful in recovering many items not originally reported, such as common additions to foods (e.g., butter on toast) and eating occasions not originally reported

(e.g., snacks and beverage breaks). However, interviewers should be provided with standardized neutral probing questions so as to avoid leading the respondent to specific answers when the respondent really does not know or remember.

The current state-of-the-art 24-hour dietary recall protocol in the United States is the U.S. Department of Agriculture's (USDA) Automated Multiple-Pass Method (AMPM) [82,83], which is used in the U.S. National Health and Nutrition Examination Survey (NHANES). The AMPM five-pass method consists of (1) an initial "quick list," in which the respondent reports all the foods and beverages consumed, without interruption from the interviewer; (2) a forgotten foods list of nine food categories commonly omitted in 24-hour recall reporting; (3) time and occasion, in which the time each eating occasion began and what the respondent would call it are reported; (4) a detail pass, in which probing questions ask for more detailed information about the food and the portion size, in addition to review of the eating occasions and times between the eating occasions; and (5) final review, in which any other item not already reported is asked [82,83]. In addition, a two-dimensional Food Model Booklet [84], developed from USDA research, is used in the NHANES in order to facilitate more accurate portion size estimation. A 24-hour recall interview using the multiple-pass approach typically requires between 30 and 45 minutes.

Data processing software systems are currently available in most developed countries, allowing direct coding of most foods reported during the interview. This is highly efficient with respect to processing dietary data, minimizing missing data, and standardizing interviews [85,86]. If direct coding of the interview is done, methods for the interviewer to easily enter those foods not found in the existing database should be available, and appropriate use of these methods should be reinforced by interviewer training and quality control procedures.

A huge technological advance in 24-hour dietary recall methodology is the development of automated self-administered data collection instruments [76,78–80,87–91]. These systems vary in their design, inclusion of probes regarding details of foods consumed and possible additions and omissions, the approach to asking about portion size, and the number of foods in their databases. The Automated Self-Administered 24-hour dietary recall (ASA24) developed at the National Cancer Institute (NCI) [76,90,91] incorporates many elements of the AMPM 24-hour interview developed by USDA [82]. Prompts used in the AMPM are asked in the program. Portion sizes are reported using digital photographs depicting up to eight sizes as portion size aids [91]. The system uses the most current USDA survey database [92] to allow automated coding and processing and ultimately estimation of nutrient and food group intakes. The ASA24 system is freely available for web or mobile phone administration [76]. Such automated tools make feasible the collection of high-quality dietary data in large-scale population research. Automated self-administered recalls have been compared to interviewer-administered recalls. One study in adolescents found that differences between interviewer- and self-administered recalls were minimal [80]. A feeding study of 86 adults found that the AMPM and the ASA24 were comparable in their agreement with observed intake [93]. Additionally, a large field study in 1083 adults found that nutrient and food group intakes estimated from AMPM and ASA24 recalls were comparable, and that the ASA24 was preferred over the AMPM by 70% of the participants [94].

There are many advantages to the 24-hour recall. When an interviewer administers the tool and records the responses, literacy of the respondent is not required. For self-administered versions, literacy can be a constraint. Because of the immediacy of the recall period, respondents are generally able to recall most of their dietary intake. Because there is relatively little burden on the respondents, those who agree to do 24-hour dietary recalls are more likely to be representative of the population than are those who agree to keep food records. Thus, the 24-hour recall method is useful across a wide range of populations. In addition, interviewers can be trained to capture the detail necessary so that new foods reported can be researched later by the coding staff and coded appropriately. Finally, in contrast to record methods, dietary recalls occur after the food has been consumed, and if unscheduled, reactivity is not a problem.

The main weakness of the 24-hour recall approach is that individuals may not report their food consumption accurately for various reasons related to knowledge, memory, and the interview situation. These cognitive influences are discussed in more detail in Section V.A. A potential limitation, as is true for food records, is that multiple days of recalls may be needed for the study objective. Whereas a single 24-hour recall can be used to describe the average dietary intake of a population, multiple days of recalls are needed to model estimates of the population's usual intake distributions. Multiple administrations of 24-hour recalls also allow more precise estimation of relationships with other factors (see Section V.G).

As with other self-report instruments, relative validation, for example, comparing 24-hour recalls with food records, is not particularly useful. The validity of the 24-hour dietary recall has been studied by comparing respondents' reports of intake either with intakes unobtrusively recorded/weighed by trained observers or with recovery biomarkers. Numerous observational studies of the performance of the 24-hour recall have been conducted with

children (see Section IV.C). In studies of adults, group mean nutrient estimates from 24-hour recalls have been found to be similar to observed intakes [5,95], although respondents with lower observed intakes have tended to overreport energy and those with higher observed intakes have tended to underreport energy [95]. One observational study found energy underreporting during a self-selected eating period in both men and women, similar underreporting during a controlled diet period in men, and accurate reporting during a controlled diet period in women; underestimates of portion sizes accounted for much of the underreporting [96]. A study of adults comparing AMPM and ASA24 to observed intake found that both protocols captured about 80% of the foods and drinks actually consumed; there were few differences in nutrient and food group intakes between observed and reported for both protocols [93]. Studies with the recovery biomarkers of doubly labeled water and urinary nitrogen generally have found underreporting using 24-hour dietary recalls for energy in the range of 3−34% [22,42,79,83,97−103], with the largest two studies in adults using a multiple-pass method showing average underreporting to be between 12% and 23% [83,100]. For protein, underreporting tends to be in the range of 11−28% [97,100,101,103−107]. An analysis of data pooled from five of the larger recovery biomarker studies found an average rate of underreporting of 15% for energy and 5% for protein [108]. However, underreporting is not always found. Some studies found overreporting of energy from 24-hour dietary recalls compared to doubly labeled water in proxy reports for young children and adolescents [109,110]. In addition, it is likely that the commonly reported phenomenon of underreporting in Western countries may not occur in all cultures; for example, Harrison et al. [111] reported that 24-hour recalls collected from Egyptian women were well within expected amounts. Finally, in many studies, energy adjustment has been found to reduce error. For example, for protein density (i.e., percentage energy from protein), 24-hour dietary recalls conducted in the large biomarker studies were in close agreement or somewhat higher compared to a biomarker-based measure [54,100,101].

In past national dietary surveys using multiple-pass methods, findings suggest that energy underreporting may affect up to 15% of all 24-hour recalls [112,113]. Underreporters compared to nonunderreporters tended to report fewer numbers of foods, fewer mentions of foods consumed, and smaller portion sizes across a wide range of food groups and tended to report more frequent intakes of low-fat/diet foods and less frequent intakes of fat added to foods [112]. As was found for records, factors such as BMI, sex, social desirability, restrained eating, education, literacy, perceived health status, and race/ethnicity have been shown in various studies to be related to underreporting in recalls [48,54,62,64,83,98,106,108,112−116]. The 24-hour dietary recall is considered the least biased self-report instrument, and thus is useful for most research purposes. The NCI Dietary Assessment Primer gives extensive guidance as to its use in research studies [117].

C Food Frequency

The food frequency approach asks respondents to report their usual frequency of consumption of each food from a list of foods for a specific period. Information is collected on frequency, but little detail is collected on other characteristics of the foods as eaten, such as the methods of cooking, or the combinations of foods in meals. Many FFQs also incorporate usual portion size questions or specify portion sizes as part of each question. Overall nutrient intake estimates are derived by summing, over all foods, the products of the reported frequency of each food by the amount of nutrient in a specified (or assumed) serving of that food to produce an estimated daily intake of nutrients, dietary constituents, and food groups. In most cases, the purpose of an FFQ is to obtain a crude estimate of usual total daily intakes over a designated time period.

There are many FFQ instruments, and many continue to be adapted and developed for different populations and purposes. Among those evaluated and commonly used are the Block Questionnaires [118], the Harvard University Food Frequency Questionnaires or Willett Questionnaires [119], the Fred Hutchinson Cancer Research Center Food Frequency Questionnaire [120,121], and the NCI's Diet History Questionnaire [122], which was designed with an emphasis on cognitive ease for respondents [123,124]. FFQs have been developed for use with specific populations in the United States (e.g., African Americans, Hispanics) and throughout the world. Because of the number of FFQs available, investigators planning to use an FFQ need to carefully consider which best suits their research needs. "Brief" FFQs that assess a limited number of dietary exposures are discussed in the next section.

The appropriateness of the food list is crucial in the food frequency method. The entire breadth of an individual's diet, which includes many different foods, brands, and preparation practices, cannot be fully captured with a finite food list. Obtaining accurate reports for foods eaten both as single items and in mixtures is particularly problematic. FFQs can ask the respondent either to report a combined frequency for a particular food eaten both alone and in mixtures or to report separate frequencies for each food use. (For example, one could ask about beans eaten alone and in mixtures, or one could ask separate questions about refried beans, bean soups, beans in burritos, etc.) The first approach is cognitively complex for the respondent, but the second approach may lead to double

counting (e.g., burritos with beans may be reported as both beans and as a Mexican mixture). Often, FFQs will include similar foods in a single question (e.g., beef, pork, or lamb). However, such grouping can create a cognitively complex question (e.g., for someone who often eats beef and occasionally eats pork and lamb). Differences in definitions of the food items asked may also be problematic; for example, rice is judged to be a vegetable by many nonacculturated Hispanics living in the United States, a judgment not shared in other race/ethnic groups [125]. Finally, when a group of foods is asked as a single question, assumptions about the relative frequencies of intake of the foods constituting the group are made in the assignment of values in the nutrient database. These assumptions are generally based on information from an external study population (such as from a national survey sample) even though true eating patterns may differ considerably across population subgroups and over time.

Each quantitative FFQ must be associated with a database to allow estimation of nutrient intakes for an assumed or reported portion size of each food queried [126]. For example, the FFQ item of macaroni and cheese encompasses a wide variety of different recipes with different nutrient composition, yet the FFQ database must have a single nutrient composition profile. There are several approaches to constructing such a database. One approach uses quantitative dietary intake information from the target population to define the typical nutrient density of a particular food group category. For example, for the food group macaroni and cheese, all reports of the individual food codes reported in a population survey can be collected, and a mean or median nutrient composition (by portion size if necessary) can be estimated. Values can also be calculated by sex and age. Dietary analysis software, specific to each FFQ, is then used to compute nutrient intakes for individual respondents. These analyses are available commercially for the Block, Willett, and Fred Hutchinson FFQs, and are publicly available for the NCI Diet History Questionnaire.

In pursuit of improving the validity of the FFQ, investigators have addressed a variety of frequency questionnaire design issues, such as length, closed- versus open-ended response categories, portion size, seasonality, and time frame. Frequency instruments designed to assess total diet generally list more than 100 individual line items, many with additional portion size questions, requiring 30–60 minutes to complete. In fact, some research suggests that FFQs with even longer food lists (e.g., 200 items) may perform better than those with shorter food lists (e.g., 100 items) [127]. This raises concern about the length and its effect on response rates. Although respondent burden is a factor in obtaining reasonable response rates for studies in general, a few studies have shown that respondent burden does not seem to be a decisive factor for FFQs [124,128,129]. This tension between length and specificity highlights the difficult issue of how to define a closed-ended list of foods for a food frequency instrument. Using food record intake information, a recently described mathematical approach considers the length, coverage, and explained variance to derive an optimized food list [130]. It is suggested that this tool be used in conjunction with expert judgment from a research nutritionist.

Although the amounts consumed by individuals are considered an important component in estimating dietary intakes, it is controversial as to whether or not portion size questions should be included on FFQs [127]. Frequency has been found to be a greater contributor than serving size to the variance in intake of most foods [131,132], suggesting that the additional respondent burden of reporting serving sizes is not worthwhile. Others cite small improvements in the performance of FFQs that ask the respondents to report a usual serving size for each food [133,134]. Some incorporate portion size and frequency into one question, asking how often a particular portion of the food is consumed [135]. Although some research has been conducted to determine the best ways to ask about portion size on FFQs [123], the marginal benefit of such information in a particular study may depend on the study objective and population characteristics [136]. The ramifications of using self-reported versus standard portion sizes were illustrated in a case–control study that found different odds ratios depending on which metric was used [137].

Another design issue is the time frame about which intake is queried. Most instruments inquire about usual intakes during the past year, but others ask about the past week or month [138], depending on specific research situations. Even when intake during the past year is asked, some studies have indicated that the season in which the questionnaire is administered has an influence on reporting for the entire year [139–141].

Finally, analytical decisions are required in how food frequency data are processed. In research applications in which there are no automated quality checks to ensure that all questions are asked, decisions about how to handle missing data are needed. In particular, in self-administered situations, there are usually many initial frequency questions that are not answered. One approach is to assign null values because some research indicates that respondents selectively omit answering questions about foods they seldom or never eat [142,143]. Another approach is the imputation of frequency values for those not providing valid answers. Only a few studies have addressed this issue [144,145], and it is currently unclear whether imputation is an advance in FFQ analyses. Recently, however, paper and pencil administration has declined and has been replaced by electronic administration which, because of programmable skip patterns, greatly reduces missing data.

Strengths of the FFQ approach are that it is inexpensive to administer and process and it asks about the respondent's usual intake of foods over an extended period of time. Unlike other methods, the FFQ can be used to circumvent recent changes in diet (e.g., changes due to disease) by obtaining information about individuals' diets as recalled about a prior time period. Retrospective reports about diet nearly always use a food frequency approach. Food frequency responses are used to rank individuals according to their usual consumption of nutrients, foods, or groups of foods. Nearly all food frequency instruments are designed to be self-administered, and most are either optically scanned paper versions or administered electronically [118,120,122,146–148]. Because the costs of data collection and processing and the respondent burden have traditionally been much lower for FFQs than for multiple diet records or recalls, FFQs have been a common way to estimate usual dietary intake in large epidemiological studies.

The major limitation of the food frequency method is that it contains a substantial amount of measurement error [54,100–103,149]. Many details of dietary intake are not measured, and the quantification of intake is not as accurate as with recalls or records. Inaccuracies result from an incomplete listing of all possible foods and from errors in frequency and usual serving size estimations. The estimation tasks required for an FFQ are complex and difficult [150]. As a result, the scale for nutrient intake estimates from an FFQ may be shifted considerably, yielding inaccurate estimates of the average intake for the group. Research suggests that longer food frequency lists may overestimate whereas shorter lists may underestimate intake of fruits and vegetables [151], but it is unclear whether or how this applies to nutrients and other food groups.

Portion size of foods consumed is difficult for respondents to evaluate and is thus problematic for all assessment instruments (see Section V.D). However, the inaccuracies involved in respondents attempting to estimate usual portion size in FFQs may be even greater because a respondent is asked to estimate an average for foods that may have highly variable portion sizes across eating occasions and time periods [152].

Because of the error inherent in the food frequency approach, it is generally considered inappropriate to use FFQ data to estimate quantitative parameters, such as the mean and variance, of a population's usual dietary intake [153–158]. Although some FFQs seem to produce estimates of population average intakes that are reasonable [153,159,160], different FFQs will perform in often unpredictable ways in different populations, so the levels of nutrient intakes estimated by FFQs should best be regarded as only approximations [154]. FFQ data are usually energy adjusted and then used for ranking subjects according to food or nutrient intake rather than for estimating absolute levels of intake, and they are used widely in case–control or cohort studies to assess the association between dietary intake and disease risk [161–163]. For estimating relative risks, the degree of misclassification of subjects is more important than is the quantitative scale on which the ranking is made [164].

The definitive validity study for a food frequency–based estimate of long-term usual diet would require nonintrusive observation of the respondent's total diet over a long time. Such studies are not possible in free-living populations. One early feeding study, with three defined 6-week feeding cycles (in which all intakes were known), showed some significant differences in known absolute nutrient intakes compared to the Willett FFQ for several fat components, mostly in the direction of underestimation by the FFQ [165]. Many studies have compared food frequency estimates with those from multiple food recalls or records over a period of time (see [166] for a register of such studies). However, recalls and records cannot be considered as accurate reference instruments because they themselves have error. Validation studies of various FFQs using recovery biomarkers have found that FFQs underestimate energy intake by 11%–35% [42,48,51,54,79,97,99–103] and protein intake by up to 30% [46,47,54,97,100,101,103,167–171]. In a pooled analysis of five larger U.S. biomarker studies, FFQs underestimated energy by 28% and protein by 10% [108]. A few studies show that correlations between a biomarker for protein density constructed from both urinary nitrogen and doubly labeled water and self-reported protein density on an FFQ (kcal of protein as a percentage of total kcal) are higher than correlations between urinary nitrogen and FFQ-reported absolute protein intake [101,103,149], indicating that energy adjustment may alleviate some of the error inherent in food frequency instruments. Various statistical methods employing measurement error models and energy adjustment are used not only to assess the validity of FFQs but also to adjust estimates of relative risks for disease outcomes [54,172–182]. However, analyses indicate that correlations between an FFQ and a reference instrument, such as the 24-hour recall, may be overestimated because of correlated errors [54,101,149]. Furthermore, a few analyses comparing relative risk estimation from FFQs to dietary records [183,184] in prospective cohort studies indicate that observed relationships are attenuated with FFQs, thereby obscuring associations that might exist; however, not all analyses have found this result [185]. Some epidemiologists have suggested that the error in FFQs is a serious enough problem that more accurate methods (e.g., food records or 24-hour recalls) of assessing dietary intake in large-scale prospective studies should be considered [186–188].

Because of relatively large measurement error and bias found with FFQs, the NCI Dietary Assessment Primer suggests they be used sparingly, especially when other instruments such as 24-hour dietary recalls could be used. When FFQs are used as the main instrument, a concurrent calibration study on a subsample of the population using more accurate instruments should be included in the design [117]. See Section V.C for more discussion of calibration. Because FFQ data might be combined with recall or record data to improve estimates of intake and relative risks [188–190], the use of both instruments may be optimal [117].

D Brief Dietary Assessment Instruments

Many brief dietary assessment instruments, also known as "screeners," have been developed. These instruments can be useful in situations that do not require either assessment of the total diet or quantitative accuracy in dietary estimates. For example, a brief diet assessment of some specific dietary components may be used to triage large numbers of individuals into groups to allow more focused attention on those at greatest need for intervention or education. Measurement of dietary intake, even if imprecise, can also serve to activate interest in the respondent, which in turn can facilitate nutrition education. Brief instruments may therefore have utility in clinical settings or in situations in which health promotion and health education is the goal. In the intervention setting, brief instruments focused on specific aspects of a dietary intervention have been used to track changes in diet. However, because of concern that responses to questions of intake that directly evolve from intervention messages may be biased [191] and that these instruments lack sensitivity to detect dietary change [192], this use is not recommended. Brief instruments of specific dietary components such as fruits and vegetables have been used for population surveillance at the state or local level, for example, in the Centers for Disease Control and Prevention's (CDC) Behavioral Risk Factor Surveillance System (BRFSS) [193,194] and the California Health Interview Survey (CHIS) [195] (see Section III.A). Brief instruments have also been used to examine relationships between some specific aspects of diet and other exposures, such as in the National Health Interview Survey (NHIS) [196]. Finally, some suggest the use of brief instruments to evaluate the effectiveness of policy initiatives [195,197,198], although others question the ability of short measures to adequately evaluate dietary changes [199].

Brief instruments can be simplified or targeted FFQs, questionnaires that focus on specific eating behaviors other than the frequency of intake of specific foods, or daily checklists. Complete FFQs typically contain 100 or more food items to capture the range of foods contributing to the many different nutrients in the diet. If an investigator is interested only in estimating the intake of a single nutrient or food group, however, then far fewer foods need to be assessed. Often, only 15–30 foods might be required to account for most of the intake of a particular food component [200,201].

Numerous short questionnaires using a food frequency approach have been developed and compared with multiple days of dietary records, 24-hour recalls, complete FFQs, and/or biological indicators of diet. The NCI has developed a Register of Validated Short Dietary Assessment Instruments [202], which contains descriptive information about short instruments and their validation studies and publications, as well as copies of the instruments when available. To be included, publications are required to be in English language peer-reviewed journals and published since January 1998. Currently, the register includes nearly 140 instruments assessing more than 30 dietary factors from 31 different countries. Instruments in the register may be searched by dietary factors, questionnaire format, and number of questions. Descriptive information about the validation study includes the reference tool, the study population (age, sex, and race/ethnicity), and the geographical location.

Much of the focus in brief instrument development has been on fruits and vegetables and on fats. Some work has addressed other food components that are found in relatively few foods, such as calcium, added sugars, soy, phytoestrogens, and heterocyclic amines [202].

1 Brief Instruments Assessing Fruit and Vegetable Intake

Food frequency-type instruments to measure fruit and vegetable consumption range from a single overall question to 45 or more individual questions [203–207]. An early 7-item tool developed by the NCI and private grantees for NCI's 5 A Day for Better Health Program effort was used widely in the United States [208–210]. This tool was similar to one used in CDC's BRFSS [193,211,212]. Validation studies of the BRFSS and 5 A Day brief instruments to assess fruit and vegetable intake suggested that without portion size adjustments, they often underestimated actual intake [203,208,212–214]. Using cognitive interviewing findings (see Section V.A), NCI revised the tool, including adding portion size questions; some studies indicate improved performance [215] and utility in surveillance studies. However, its performance in community interventions was mixed. In six of eight site/sex comparisons, fruit and vegetable consumption was significantly overestimated in relation to results from multiple 24-hour recalls [216]. More important, the screener indicated change in consumption in both men and women when none was

seen with the 24-hour recalls [217]. The BRFSS fruit and vegetable screener used in 2011−15 in odd years [193] assessed intake of solid fruit and 100% fruit juice and subgroups of vegetables that were particularly relevant to 2010 Dietary Guidelines for Americans [218]. Intake estimates from the 2011 and 2013 assessments with the new tool have been reported [194,219]. The instrument is being redesigned, using questions developed at NCI.

2 Brief Instruments Assessing Fat Intake

The MEDFICTS (meats, eggs, dairy, fried foods, fat in baked goods, convenience foods, fats added at the table, and snacks) questionnaire, initially developed to assess adherence to low total fat (<30% energy from fat) and saturated fat diets [219], asks about frequency of intake and portion size of 20 individual foods that are major food sources of fat and saturated fat in the U.S. diet. Its initial evaluation showed high correlations with dietary records [219]. In addition to the cross-sectional studies, the MEDFICTS underestimated percentage calories from fat; it was effective in identifying very high-fat intakes but was not effective in identifying moderately high-fat diets [220] or correctly identifying low-fat diets [221]. The number of mixtures reported on an FFQ (e.g., pizza and macaroni and cheese), which were not specifically included in the MEDFICTS tool, was negatively related to its predictive ability [221]. In a longitudinal setting, positive changes in the MEDFICTS score have been correlated with improvements in serum lipids and waist circumference among cardiac rehabilitation patients [222]. The instrument has been adapted for other populations with varying success [221,223]. Other fat screeners have been developed to preserve the between-person variability of intake [224−226]—that is, to focus on the fat sources that most distinguish differences in fat intake among individuals or groups. A 20-item screener was developed and tested at the German site of European Prospective Investigation into Cancer and Nutrition correlated with 7-day dietary records ($r = 0.84$) and a complete FFQ ($r = 0.82$) [224,225]. A 16-item percentage energy from fat screener had a correlation of 0.6 with 24-hour recalls in an older U.S. population [226]. However, its performance in overweight African-American women was poorer (mean of 33.0% vs 35.5% energy from fat for screener vs 24-hour recall) [227]. Its performance in an intervention study of adults varied by site [228].

Often, dietary fat reduction interventions are designed to target specific food preparation or consumption behaviors rather than frequency of consuming specific foods. Such behaviors might include trimming the fat from red meats, removing the skin from chicken, or choosing low-fat dairy products. Many questionnaires have been developed in various populations to measure these types of dietary behaviors [229−238], and many have been found to correlate with fat intake estimated from other more detailed dietary instruments [239,240] or with blood lipids [233,241,242]. In addition, some studies have found that changes in dietary behavior scores have correlated with changes in blood lipids [234,241,243]. The instrument has been updated and modified for use in different settings and populations [242,244,245]. A modification tested in African-American adolescent girls had a relatively low correlation ($r = 0.31$) with multiple 24-hour recalls [246]. In another modification developed for African-American women [247], a subset of 30 items from the SisterTalk Food Habits Questionnaire correlated with change in BMI ($r = -0.35$) as strongly as did the original 91 items ($r = -0.36$) [248].

3 Brief Multifactor Instruments

Recognizing the utility of assessing a few dimensions of diet simultaneously, several multifactor short instruments have been developed and evaluated. For example, PrimeScreen is composed of 18 FFQ items asking about consumption of fruits and vegetables, whole and low-fat dairy products, whole grains, fish and red meat, and sources of saturated and trans-fatty acids. The average correlation with estimates from a full FFQ over 18 food groups was 0.6 and over 13 nutrients was also 0.6 [249]. The NCI developed a dietary screener administered in the 2009−10 NHANES that included 28 items addressing consumption of fruits and vegetables, whole grains, added sugars, dairy, fiber, calcium, red meats, and processed meats [250]. This screener was also used in the 2010 and 2015 NHIS Cancer Control Supplement.

Some multicomponent behavioral questionnaires have also been developed. For example, Schlundt et al. [251] developed a 51-item Eating Behavior Patterns Questionnaire targeted at assessing fat and fiber consumption among African-American women. Newly incorporated in this questionnaire were questions to reflect emotional eating and impulsive snacking.

Some instruments combine aspects of food frequency and behavioral questions to assess multiple dietary patterns. For example, the Rapid Eating and Activity Assessment for Patients is composed of 27 items assessing consumption of whole grains, calcium-rich foods, fruits and vegetables, fats, sugary beverages and foods, sodium, and alcohol. When compared to dietary records, correlations were 0.49 with the original Healthy Eating Index (HEI) [252], a measure of overall diet quality, and moderately high (range of $r = 0.33−0.55$) for HEI subscores of fat, saturated fat, cholesterol, fruit, and meat. Correlations for other HEI subscores for sodium, grains, vegetables, and dairy were low (range of $r = 0.03−0.27$) [253].

Because the cognitive processes for answering food frequency-type questions can be complex, some attempts have been made to reduce respondent burden by creating brief instruments with questions that require only "yes−no" answers. This approach has been applied as a modification of the 24-hour recall [254]. These "targeted" 24-hour recall instruments aim to assess particular foods, not the whole diet [71,255−257]. They present a precoded close-ended food list and ask whether the respondent ate each food on the previous day; portion size questions may also be asked. For example, a web-administered checklist has been developed to measure the Dietary Approaches to Stop Hypertension diet. It includes a listing of foods grouped into 11 categories, and it includes serving size information [258].

4 Limitations of Brief Instruments

The brevity of these instruments and their correspondence with dietary intake as estimated by more extensive methods create a seductive option for investigators who would like to measure dietary intake at a low cost. Although brief instruments have many applications, they have several limitations. First, they do not capture information about the entire diet. Most measures are not quantitatively meaningful and, therefore, estimates of dietary intake for the population usually cannot be made. Even when measures aim to provide estimates of total intake, the estimates are approximations and have large measurement error. Finally, the specific dietary behaviors found to correlate with dietary intake in a particular population may not correlate similarly in another population or even in the same population at another time period. For example, a brief instrument developed to assess fast-food and beverage consumption in a primarily white, adolescent population [259] was not useful in an overweight Latina adolescent population [260]. Investigators should carefully consider the needs of their study and their own population's dietary patterns before choosing an "off-the-shelf" instrument designed to briefly measure either food frequency or specific dietary behaviors. Because of these limitations, the NCI Dietary Assessment Primer recommends that short instruments be used sparingly and when used, to be calibrated to a more accurate instrument such as 24-hour dietary recalls [117]. See Section V.C for more discussion on calibration.

E Diet History

The term *diet history* is used in many ways. In the most general sense, a dietary history is any dietary assessment that asks the respondent to report about past diet. Originally, as coined by Burke, the term *dietary history* referred to the collection of information not only about the frequency of intake of various foods but also about the typical makeup of meals [261,262]. Many now imprecisely use the term dietary history to refer to the food frequency method of dietary assessment. However, several investigators have developed diet history instruments that provide information about usual food intake patterns beyond simply food frequency data [263−266]. Some of these instruments characterize foods in much more detail than is allowed in food frequency lists (e.g., preparation methods and foods eaten in combination), and some of these instruments ask about foods consumed at every meal [265,267]. The term diet history is therefore probably best reserved for dietary assessment methods that are designed to ascertain a person's usual food intake in which many details about characteristics of foods as usually consumed are assessed in addition to the frequency and amount of food intake.

The Burke diet history included three elements: a detailed interview about usual pattern of eating, a food list asking for amount and frequency usually eaten, and a 3-day dietary record [261,262]. The detailed interview (which sometimes includes a 24-hour recall) is the central feature of the Burke dietary history, with the food frequency checklist and the 3-day diet record used as cross-checks of the history. The original Burke diet history, which requires administration by an interviewer, has not often been exactly reproduced because of the effort and expertise involved in capturing and coding the information. However, many variations of the Burke method have been developed and used in a variety of settings [263−266,268−272]. These variations attempt to ascertain the usual eating patterns for an extended period of time, including type, frequency, and amount of foods consumed; many include a cross-check feature [273,274].

Some diet history instruments have been automated and adapted for self-administration, sometimes with audio, thus eliminating the need for an interviewer to ask the questions [24,265,275]. Other diet histories have been automated but still continue to be administered by an interviewer [276,277]. Short-term recalls or records are often used for validation or calibration rather than as a part of the tool.

The major strength of the diet history method is its assessment of meal patterns and details of food intake rather than intakes for a short period of time (as in records or recalls) or only frequency of food consumption. Details of the means of preparation of foods can be helpful in better characterizing nutrient intake (e.g., frying vs baking), as well as exposure to other factors in foods (e.g., charcoal broiling). When the information is collected separately for each meal, analyses of the joint effects of foods eaten together are possible (e.g., effects on iron absorption of concurrent intake of tea or foods containing vitamin C). Although a meal-based approach

often requires more time from the respondent than does a food-based approach, it may provide more cognitive support for the recall process. For example, the respondent may be better able to report total bread consumption by reporting bread as consumed at each meal.

A weakness of the approach is that respondents are asked to make many judgments about both the usual foods consumed and the amounts of those foods eaten. These subjective tasks may be difficult for many respondents. Burke cautioned that nutrient intakes estimated from these data should be interpreted as relative rather than absolute. All of these limitations are also shared with the food frequency method. The meal-based approach is not useful for individuals who have no particular eating pattern and may be of limited use for individuals who "graze" (i.e., eat throughout the day rather than at defined mealtimes). The approach, when conducted by interviewers, requires trained nutrition professionals and is thus costly. Finally, the diet history as a method is not well standardized, and thus methods differ from each other and are difficult to reproduce, making comparisons across studies difficult.

Relative to other assessment approaches, few validation studies of diet history questionnaires using biological markers as a basis of comparison have been conducted. The studies found that reported mean energy intakes using the diet history approach in selected small samples of adults were underestimated in the range of 2−23% compared to energy expenditure as measured by doubly labeled water [278−281]. Generally, underreporting of protein, compared to urinary nitrogen, was less than that for energy and only sometimes significantly different [279,281−283]. These results have also been seen in children [284], adolescents [285,286], and the elderly [264]. Because of small sample sizes in these studies, few were able to examine characteristics related to underreporting, and their results were mixed, with some finding more underreporting with higher BMI [283,284] and others finding no relationship [264,280,287]. Although the diet history approach was extensively used as the main study instrument in European cohorts initiated in the 1990s, the approach is seldom used now in new cohort studies as other approaches have evolved. The approach is sometimes used as a reference instrument [288−290].

F Blended Instruments/Combined Instruments

Better understanding of various instruments' strengths and weaknesses has led to creative blending of instruments with the goal of maximizing the strengths of each instrument. For example, a record-assisted 24-hour recall has been used in several studies with children [291,292]. The child keeps notes of what he or she has eaten and then uses these notes as memory prompts in a later 24-hour recall. A mobile phone food record app that includes before and after meal photographs with text entry has been tested in adolescents [293].

Analytical methods for using information from two different instruments are available. For example, Thompson et al. [294] combined information from a series of daily checklists (i.e., precoded record) with frequency reports from an FFQ to form checklist-adjusted estimates of intake. In an evaluation of this approach, agreement with 24-hour recalls improved for energy and protein but was unchanged for protein density [294]. A two-part statistical model developed by NCI uses information from two or more 24-hour recalls, allowing for the inclusion of daily frequency estimates derived from a food propensity questionnaire (a frequency questionnaire that does not ask about portion size), as well as other potentially contributing characteristics (e.g., age and race/ethnicity), as covariates [295]. Frequency information contributes to the model by providing additional information about an individual's propensity to consume a food, and is particularly useful for episodically consumed foods and nutrients [296]. The recalls, however, provide information about the nature and amount of the food consumed. Such methods are used to better measure usual intakes (see Section V.G). Several approaches consisting of multiple dietary assessment instruments are available to estimate associations between diet and disease. A prominent use is to calibrate a frequency questionnaire completed by all study subjects with information from a more accurate instrument, such as a 24-hour recall, completed by a subset. See Section V.C for more discussion of calibration. Carroll et al. [188] explored the number of days of 24-hour recall required to estimate associations between diet and disease in a cohort study and whether an FFQ, in addition, is beneficial. They concluded that for most nutrients and foods, 4−6 nonconsecutive days of 24-hour recall and an FFQ are optimal. The combination of FFQ and multiple 24-hour recalls was superior in estimating some nutrients and foods, especially for episodically consumed foods. Finally, the addition of biomarker information to self-reported dietary information has been shown to increase accuracy and statistical power to estimate associations between diet and disease [297,298].

Table 1.1 summarizes the important characteristics of the main self-report dietary assessment methods.

III DIETARY ASSESSMENT IN DIFFERENT STUDY DESIGNS

The choice of the most appropriate dietary assessment method for a specific research question requires careful

TABLE 1.1 Comparison of Self-Report Dietary Assessment Methods by Important Characteristics

	Dietary Record	24-Hour Recall	FFQ	Diet History	Screener
Type of Information Attainable					
Detailed information about foods consumed	X	X		X	
General information about food groups consumed			X		X
Meal-specific details	X	X		X	
Scope of Information Sought					
Total diet	X	X	X	X	
Specific components					X
Time Frame Asked					
Short term (e.g., yesterday, today)	X	X		X	
Long term (e.g., last month, last year)			X	X	X
Adaptable for Diet in Distant Past					
Yes			X	X	X
No	X	X			
Cognitive Requirements					
Measurement or estimated recording of foods and drinks as they are consumed	X				
Memory of recent consumption		X		X	
Ability to make judgments of long-term diet			X	X	X
Potential for Reactivity					
High	X				
Low		X	X	X	X
Time Required to Complete					
<15 minutes					X
>20 minutes	X	X	X	X	
Suitable for Cross-Cultural Comparisons Without Instrument Adaptation					
Yes	X	X		X	
No			X	X	X

consideration. The primary research question must be clearly formed, and questions of secondary interest should be recognized as such. Projects can fail to achieve their primary goal because of too much attention to secondary goals. The choice of the most appropriate dietary assessment tool depends on many factors. Questions that must be answered in evaluating which dietary assessment tool is most appropriate for a particular research need include the following [162]: (1) Is information needed about foods, nutrients, other food components, or specific dietary behaviors? (2) Is the focus of the research question on describing intakes using estimates of average intake, and does it also require distributional information? (3) Is the focus of the research question on describing relationships between diet and health outcomes? (4) What level of accuracy and precision is needed? (5) What time period is of interest? (6) What are the research constraints in terms of money, interview time, staff, and respondent characteristics?

The NCI Dietary Assessment Primer conceptualizes research questions into four categories: to describe a population's dietary intake; to examine associations between diet as an independent variable and another variable; to examine associations between an independent

variable and diet as a dependent variable; and to evaluate the effect of an intervention on dietary intake. The role of measurement error in tool selection for each research objective is discussed in depth [117].

A Cross-Sectional Surveys

One of the most common types of population studies is the cross-sectional survey, a set of measurements of a population at a particular point in time. Such data can be collected solely to describe a particular population's intake. Alternatively, data can be used for surveillance at the national, state, and local levels as the basis for assessing risk of deficiency, toxicity, and overconsumption; to evaluate adherence to dietary guidelines and public health programs; and to develop food and nutrition policy. Cross-sectional data also may be used for examining associations between current diet and other factors including health. However, caution must be applied in examining many chronic diseases believed to be associated with past diet because the currently measured diet is not necessarily related to past diet. If the study objective requires quantitative estimates of intake, the 24-hour recall and possibly the food record instruments are recommended [117]. Less detailed instruments, such as FFQs or behavioral indicators, may be appropriate when qualitative estimates on limited exposures are sufficient—for example, frequency of consuming sugar-sweetened beverages and frequency of eating from fast-food restaurants.

1 Surveillance/Monitoring

When measurements are collected on a sample at two or more times, the data can be used for purposes of monitoring dietary trends. To assess trends in intakes over time, it would be ideal for the dietary surveillance data collection methods, sampling procedures, and food composition databases to be similar from survey to survey. As a practical matter, however, this is difficult, and the benefits of trend analysis may not outweigh the benefits of improving the methods over time. The dietary assessment method used consistently throughout the years in U.S. national dietary surveillance is the interviewer-administered 24-hour recall. However, recall methodology has improved over time based on cognitive research, the addition of multiple interviewing passes, standardization of probes, automation of the interview, and automation of the coding. The availability of automated self-administered 24-hour recall instruments may lead to further changes in methodology.

Another issue that affects the assessment of trends over time is changes in the nutrient or food grouping databases and specification of default foods. Changes in the food supply are reflected in additions or subtractions to food composition databases, whereas changes in consumption trends may lead to subsequent reassignment of default codes for foods not fully specified in 24-hour recalls or records (e.g., when type of milk is not specified, the default code is now 2% milk as opposed to whole milk in the past). Food composition databases, too, are modified over time because of true changes in food composition, improved analytic methods for particular nutrients, or inclusion of information for new dietary components. Since 1999, the major cross-sectional surveillance survey in the United States has been the NHANES [299]. This survey is conducted by the National Center for Health Statistics. The dietary component of the survey, called "What We Eat in America" [75], consists of 24-hour recalls collected using the USDA's AMPM (see Section II.B). The USDA also processes and analyzes the data. The 24-hour recalls in NHANES query the intake of dietary supplements as well as foods and beverages. Since 2003–04, NHANES has conducted two 24-hour dietary recalls on each respondent, allowing for estimation not only of average usual intake but also of the distributions of usual intake of the dietary components (see Section V.G).

NHANES provides high-quality dietary intake data at the national level, but these data are of limited use for state and local researchers planning and evaluating their programs and policies [300]. Collection of state and local data is often constrained by lack of resources or interview time, leading to the frequent use of less expensive brief instruments. For example, the CDC has used telephone-administered brief instruments to periodically assess fruit and vegetable intake within the BRFSS [193]. The California Department of Public Health, in its California Dietary Practices Survey, has assessed dietary practices among adults biennially since 1989 [301]. The CHIS used telephone-administered brief instruments to assess fruit and vegetable intake in 2001, 2005, and 2009 [195].

B Case–Control (Retrospective) Studies

A case–control study design classifies individuals with regard to current disease status (as cases or controls) and relates this to past (retrospective) exposures. In etiologic research, information about diet before onset of disease is needed. Dietary assessment methods that focus on current behavior, such as the 24-hour recall, are obviously not useful in retrospective studies of long past diet. The food frequency and diet history methods are the only viable choices for case–control (retrospective) studies.

In any food frequency or diet history interview, the respondent is not asked to recall specific memories of each eating occasion but, rather, to respond on the basis of general perceptions of how frequently he or she ate a food. In case–control studies, the relevant period is often the year before diagnosis of disease or onset of symptoms or at particular life stages, such as adolescence and

childhood. Thus, in assessing past diet, an additional requirement is to orient the respondent to the appropriate time period.

The validity of recalled diet from the distant past is difficult to assess because definitive recovery biomarker information (e.g., doubly labeled water, urinary nitrogen) is not available for large samples from long ago. Instead, relative validity and long-term reproducibility of various FFQs have been assessed in various populations by asking participants from past dietary studies to recall their diet from that earlier time [302−304]. These studies have found that correlations between past and current reports about the past vary by nutrient and by food group [135,305], with higher correspondence for very frequently consumed and rarely consumed foods compared to that for foods consumed moderately often [305,306]. Evidence suggests that correspondence between past and recalled past decreases with the length of time between reports [302,307]. In particular, retrospective reports of diet in adolescence after long recall periods (i.e., >30 years) have shown little correspondence with the original reports [308−310]. Maternal reports about diets of their children in early childhood or adolescence and siblings reports of each other's diets in adolescence have also shown low correspondence with the original reports [310,311].

Correspondence of retrospective diet reports with the diet as measured in the original study usually has been greater than the correspondence of current diet with past diet. This observation implies that if diet from years in the past is of interest, it is usually preferable to ask respondents to recall it than to consider current diet as a proxy for past diet. Nonetheless, the current diets of respondents may affect their retrospective reports about past diets. In particular, retrospective diet reports from seriously ill individuals may be biased by recent dietary changes [302,312]. Some studies of groups in whom diet was previously measured indicate no consistent differences in the accuracy of retrospective reporting between those who recently became ill and others [313,314]. However, in two of three studies that have compared baseline prospective dietary information to later retrospective recall of the earlier diet, the correspondence of the information differed between those who later became cases and controls, introducing attenuation into risk estimates [310,315,316].

C Cohort (Prospective) Studies

In a cohort study design, exposures of interest are assessed at baseline and possibly at later times in a group (cohort) of people and disease outcomes occurring over time (prospectively) are then related to the baseline exposure levels. For many chronic diseases, large numbers of individuals need to be followed for years before enough new cases with that disease accrue to have adequate power for statistical analyses. A broad assessment of diet is usually desirable in prospective studies because many dietary exposures and many disease end points will ultimately be investigated, and areas of interest may not even be recognized at the beginning of a cohort study.

In order to relate diet at baseline prior to disease to the eventual occurrence of disease, a measure of the usual intake of foods (see Section V.G) by study subjects is needed. Multiple dietary recalls, multiple records, diet histories, and food frequency methods have all been used effectively in prospective studies. Cost and logistic issues have favored food frequency methods because many prospective studies require thousands of respondents. However, because of concern about significant measurement error and attenuation attributed to the FFQ [183,186,187,317−320], other approaches are being considered. One approach is the use of multiple automated self-administered 24-hour recall instruments (see Section II.B). Another approach is collecting multiple days of dietary records at baseline, with later coding and analysis of records for those respondents selected for analysis, using a nested case−control design [321,322]. The incorporation of emerging technological advances, such as mobile phones, in obtaining dietary records increases the feasibility of such approaches in prospective studies.

If using an FFQ as the main instrument in the cohort, it is desirable to include multiple recalls or records in representative subsamples of the population (preferably before beginning the study) to construct or modify the food frequency instrument and to calibrate it (see Section V.C). Information on the foods consumed could be used to ensure that the FFQ includes the major food sources of key nutrients, with appropriate portion size categories. Because the diets of individuals change over time, it is desirable to measure diet throughout the follow-up period rather than just at baseline. If diet is measured repeatedly over years, repeated calibration is also desirable. Information from calibration studies can be used for three purposes: to assist in study design, such as the sample size needed [164]; to calibrate values from the food frequency tool to values from the recalls/records [180]; and to determine the degree of attenuation/measurement error in the estimates of association observed in the study (e.g., between diet and disease) [175,178,180,182,323−327] (see Section V.C). Some research indicates that an optimal approach to dietary assessment in prospective studies may be the use of both multiple recalls or records and FFQs [188]. The FFQ can be particularly useful in contributing information about episodically consumed foods.

D Intervention Studies

Dietary intervention study designs usually consist of measures of interest for at least two time periods (typically, before and after intervention), and for at least two groups

of participants, those receiving the intervention and those not (i.e., controls). Intervention studies range from relatively small, highly controlled, clinical studies of targeted participants to large trials of population groups.

The need for careful planning and formative research in designing useful community dietary intervention trials has been described [328]. A critical element is the existence of evidence that a particular intervention would create a measurable change in a particular group and setting. Intentional behavior change is a complex and sequential phenomenon, as has been shown for tobacco cessation [329], and this is also true for dietary change [330].

Interventions that aim to change the existing diet may use dietary assessment for two purposes: (1) initial screening for inclusion (or exclusion) into the study and (2) baseline measurement against which dietary changes resulting from the intervention are assessed. Not all intervention trials require initial screening. For those that do, screening can be performed using very detailed instruments or less burdensome instruments. For example, food frequency instruments were used in the Women's Health Trial [331] and in the Women's Health Initiative Dietary Modification Trial [332] to identify groups with high fat intake and thus determine eligibility.

Measurement of the effects of a dietary intervention requires a valid measure of change from baseline to the conclusion of the intervention period, and often, postintervention to assess the durability of any change. Dietary interventions that are expected to change an objective marker, for example, weight or blood lipids, are relatively straightforward to measure and analyze. However, if evaluation of the intervention requires measurement of change in self-reported diets, the task is complex, due to many possible biases.

Although not intending to be deceptive, some respondents may tend to report what they think investigators want to hear, leading to social desirability [333] and social approval [334] biases. Because of their greater subjectivity, behavioral questions, short instruments, and the food frequency method may be more susceptible to social desirability biases than the 24-hour recall method [73,191]. On the other hand, repeated measurement may lead to greater awareness of diet and enhanced reporting skills and thus may enhance accuracy [335]. Dietary records and scheduled 24-hour recalls are vulnerable to reactivity bias. If assessment is by 24-hour recalls, unannounced administration would avoid reactivity but possibly at the expense of participation as successful contact may be more difficult (and expensive). Most importantly, the potential for differential misreporting of diet between study groups (whether the misreporting in each group is similar or different) can affect the integrity of the results. Repeated measures of diet among study subjects can reflect reporting bias in the direction of the change being promoted [336].

Some work has been done to evaluate the use of self-report dietary assessment methods to measure dietary changes [245,336]. Researchers have found that dietary records and scheduled 24-hour recalls are associated with changed eating behavior during the record days and less correspondence with biological measures [337] and expected weight change [338], and increased underreporting [339]. One study using dietary screeners and a reference measure of multiple nonconsecutive unannounced 24-hour recalls found that change in fruit and vegetable intake in the intervention group was overestimated relative to the control group [217]. However, in the same study, a fat screener and the 24-hour recalls were consistent in finding no change in percentage energy from fat in the two groups [340]. Because of resource constraints and respondent burden, large intervention studies have often relied on less precise measures of diet, including FFQs and brief instruments. However, resource constraints may be less relevant with the availability of automated self-administered 24-hour dietary recall instruments and less burdensome dietary records.

Because self-reports of diet are subject to differential response bias in the context of an intervention study [335,336], an independent objective assessment of dietary change should be considered. For example, food availability and/or sales in worksite cafeterias, school cafeterias, or vending machines could be monitored. One such method useful in community-wide interventions is monitoring food sales [341]. Often, cooperation can be obtained from food retailers [342]. However, because the number of food items may be large, it may be possible to monitor only a small number, and the large effects on sales of day-to-day pricing fluctuations should be carefully considered. Another method to consider is measuring changes in biomarkers of diet, such as serum carotenoids [335,343] or serum cholesterol [344]. Consistency of changes in self-reported diet and appropriate biomarkers provides further evidence for real changes in the diet. Finally, social desirability biases could be measured and the resulting scales incorporated into intervention analyses. See Chapter 10, Nutritional Intervention: Lessons from Clinical Trials, and Chapter 11, Biomarkers and Their Use in Nutrition Intervention, for more in-depth discussions of the evaluation of diet in nutrition interventions and use of biomarkers in intervention studies, respectively.

IV DIETARY ASSESSMENT IN SPECIAL POPULATIONS

A Respondents Unable to Self-Report

In many situations, respondents are unavailable or unable to report about their diets. Dietary assessment in young children relies on surrogate reports. In case–control

studies, surrogate reports may be obtained for cases who have died or who are too ill to interview. Although the accuracy of surrogate reports has not been examined using the recovery biomarkers of doubly labeled water or urinary nitrogen, comparability of reports by surrogates and subjects has been studied with the goal that surrogate information might be used interchangeably with information provided by subjects [345]. Common sense indicates that individuals who know most about a subject's lifestyle would make the best surrogate reporters [346]. Adult siblings provide the best information about a subject's early life, and spouses or children provide the best information about a subject's adult life. When food frequency instruments are used, the level of agreement between subject and surrogate reports of diet varies with the food and possibly with other variables, such as number of shared meals, interview situation, case status, and sex of the surrogate reporter. Mean frequencies of use computed for individual foods and food groups between surrogate reporters and subject reporters tend to be similar [347–349], but agreement is much lower when detailed categories of frequency are compared. Several studies have shown that agreement is better for alcoholic beverages, coffee, and tea than for foods.

When subjects themselves report intakes in the extremes of a distribution, their surrogates seldom report intakes in the opposite extreme, although the surrogates tend to report intakes in the middle of the distribution [350]. This may limit the usefulness of surrogate information for analyses that rely on accurate ranking. Furthermore, the quality of surrogate reports between spouses of deceased subjects and spouses of surviving subjects may differ substantially [351]. Thus far, however, little evidence suggests that dietary intakes are systematically overreported or underreported depending on the case status of the subject [352–354]. Nonetheless, use of surrogate respondents should be minimized for obtaining dietary information in analytical studies. When used, analyses excluding the surrogate reports should be done to examine the sensitivity of the reported associations to possible errors or biases in the surrogate reports. If planning a study using surrogate reports, sample size should be inflated to account for higher incidence of missing data, inability to recruit surrogates for some number of cases, and reduced precision of dietary estimates.

B Minority Populations

The widespread use of many "ethnic" foods in the United States throughout the population and the increasing diversity of the population have broadened the food composition databases and food lists used for the general population. Nonetheless, special modifications may be needed in dietary assessment methods when the study population is composed of individuals whose cuisine or cooking practices are not adequately represented in the instrument and/or database [355]. If the method requires an interview, interviewers of the same ethnic or cultural background are preferable so that dietary information can be more effectively communicated. If dietary information is to be quantified into nutrient estimates, examination of the nutrient composition database is necessary to ascertain whether ethnic foods are included and whether those foods and their various preparation methods represent those consumed by the target population [356]. It is also necessary to examine the recipes and assumptions underlying the nutrient composition of certain ethnic foods. Some very different foods may be called the same name, or identical foods may be called by different names [357,358]. For these reasons, it may be necessary to obtain detailed recipe information for all ethnic mixtures reported.

To examine the suitability of the initial database, preliminary information about typical diets should be collected from individuals in the minority groups. This information could come from recalls or records with accompanying interviews or from focus group interviews. These interviews should focus on the foods eaten and the ways in which foods are prepared in that culture. Recipes and alternative names of the same food should be collected, and field interviewers should be familiarized with the results of these focus groups. Recipes and food names that are relatively uniform should be included in the nutrient composition database. Even with these modifications, it may be preferable for the field interviewers to collect detailed descriptions of ethnic foods reported rather than to directly code these foods using preselected lists most common in computer-assisted methods. This would prevent the detail of food choice and preparation from being lost by a priori coding.

USDA continues to incorporate new foods into the National Nutrient Database for Standard Reference (SR) as does the University of Minnesota Nutrient Database System (see Section V.F). If a newly reported food is not available in the food composition database being used, a default code that is thought to closely mirror the nutrient composition of the new food can be used.

Use of FFQs developed for the majority population may be suboptimal for many individuals with different eating patterns. Many individuals consume both foods common in the mainstream culture and foods that are specific to their own culture. Modification of the existing food list can be accomplished through expert judgment, qualitative interviews with the target population [359], and/or examination of the frequency of reported foods in the population from a set of dietary records or recalls. For example, FFQs for Alaska Natives [360], Hispanics [361,362], and African Americans in the southern United States [363] have been developed using these approaches.

In addition to the food list, however, there are other important issues to consider when adapting existing FFQs for use in other populations. The relative intake of different foods within a food group line item may differ, thus requiring a change in the nutrient database associated with each line item. For example, Latino populations may consume more tropical fruit nectars and less apple and grape juice than the general U.S. population and therefore would require a different nutrient composition standard for juices. In addition, the portion sizes generally used may differ [364]. For example, rice may be consumed in larger quantities in Latino and Asian populations; the amount attributed to a large portion for the general population may be substantially lower than the amount typically consumed by Latino and Asian populations. Adaptation of an existing FFQ considering all of these factors has been done for an elderly Puerto Rican population [365], for white and African-American adults in the Lower Mississippi Delta [366], and for the Hawaii–Los Angeles Multiethnic Cohort Study [367]. The Southern Community Cohort Study incorporated both race/ethnicity and geographic region into its FFQ database [368].

With some populations, it may be preferable to administer an FFQ using an interviewer rather than self-administration because literacy and language barriers may limit participation in the study as well as quality of response. In addition, portion size models, which interviewers can bring to a home interview, may be preferable to portion size pictures available in a self-administered instrument [360].

The NCI Dietary Calibration/Validation Studies Register [166] can be used to search for studies using FFQs in specific race/ethnicity groups. Questionnaires aimed at allowing comparison of intakes across multiple cultures have been developed. Although some studies have found no appreciable performance differences across various race/ethnicity groups [369], most have found differences [365,367,370–374]. Understanding these differences is crucial to the appropriate interpretation of study results.

C Children

Assessing the diets of children is considered to be even more challenging than assessing the diets of adults. Children tend to have diets that are highly variable from day to day, and their food habits can change rapidly over time. Younger children are less able to recall, estimate, and cooperate in usual dietary assessment procedures than older children [375], so much information by necessity has to be obtained by surrogate reporters. Although they are more able to report, adolescents may be less motivated to give accurate reports. Baranowski and Domel [376] have posited a cognitive model of how children report dietary information.

Dietary assessment in children and adolescents has been discussed and reviewed [375,377–382]. The 24-hour recall, dietary records (including precoded checklists [8]), dietary histories, FFQs, brief instruments [383–385], and blended instruments such as a dietary record-assisted 24-hour recall [291] have all been used to assess children's intakes. The use of direct observation of children's diets has also been used extensively, most often as a reference method to compare with self-reported instruments [386,387]. As predicted from Baranowski and Domel's model, it has been found that children's estimates of portion size have large error [388], and they are less able than adults to estimate portion sizes [389] (see Section V.D). Overall, the consensus seems to be that the characteristics of different age groups call for the use of different assessment approaches [380].

For preschool-aged children, information is obtained from surrogates, usually the primary caretaker(s), typically a parent or external caregiver. If information is obtained only from one surrogate reporter, the reports are likely to be less complete. Even for periods when the caregiver and child are together, foods tend to be underestimated [390]. A "consensus" recall method, in which the child and parents report as a group on a 24-hour recall, has been shown to give more accurate information than a recall from either parent or child alone [391]. Sobo and Rock [392] describe such interviews and suggest tips for interviewers to maximize data accuracy. Food records have been used in many European population studies [393]. This approach may be acceptable, but is likely to be inappropriate for some populations. The U.S. NHANES administers 24-hour recalls to proxy reporters for children under 6 [394].

For older children, extensive research has been conducted on the self-reported 24-hour recall [395]. Baxter et al. [396] found that among fourth graders, accuracy of the 24-hour recall improves as the time between reporting and eating decreases, and meal-specific intrusions (i.e., reports of foods not consumed) are fewer in an open format interview than in a time-forward format interview (i.e., beginning at the earliest meal in the time period and working forward to the next meal). These intrusions are often associated with additional intrusions at the same meal [396]. Because accuracy of recall is greater when the time between eating and reporting is shorter, there will be differential error by meal; meals further away (e.g., at the beginning of the 24-hour recall period) will have substantially more error [397,398].

To make 24-hour recalls more feasible, self-administered automated 24-hour recall tools have been developed and tested for children [88]. An interviewer-administered 24-hour recall and a self-administered 24-hour recall using the Food Intake Recording Software System (FIRSSt) were compared to unobtrusive

observations in fourth graders. Compared to observed intake, the interviewer-administered 24-hour recall was associated with a 59% match, 17% intrusion, and 24% omission rates, whereas the automated recall was associated with a 46% match, 24% intrusion, and 30% omission rates [88]. The most recent version, FIRSSt4, is an adaptation of the ASA24, simplified for children [399,400] and is available as ASA24-Kids [76]. Particular challenges of self-administered 24-hour recalls in this age group include instigating and maintaining motivation to complete the task, and, because of difficulty in estimating portion size incorporating training for portion size estimation within the application [401]. Other web-based 24-hour recall systems have been developed especially for children and adolescents, for example, SCRAN24 in Great Britain [402], Web DASC in Denmark [403], and CANAA-W in Belgium [404]. The Synchronized Nutrition and Activity Program (SNAP), a partial recall, directs children to report the previous day's food intake by ticking the number of times they consumed each of 40 foods and 9 drinks [405]. Another approach that has been taken with school-age children is a blended instrument, the record-assisted 24-hour recall, in which the children record only the names of foods and beverages consumed throughout a 24-hour period. This information serves as a cue for the later 24-hour recall interview. The European Food Consumption Validation Project, a consortium of 13 institutes from 11 European countries, provisionally recommended a similar approach—a food recording booklet for foods eaten away from home—for school children 7−14 years old. Studies examining the validity of this approach have had mixed results [291,292,406]. For children ages 6−11, the U.S. NHANES administers 24-hour recalls to the child assisted by an adult household member. Children 12 years old and older report for themselves and may have a proxy reporter if necessary [394].

Food frequency approaches are even more challenging for children and adolescents as they are for adults. Children's diets change more quickly over time, and may also be more variable from day to day than adults. In addition, children are less able to conceptualize intake over a long period of time. The instrument itself requires adaptation of the food list, question wording and format, and portion size categories, and consequently the database for converting responses to nutrient intakes. Food frequency instruments, some web administered, have been developed and tested for use in child and adolescent populations [146,407−410]. A web-based food behavioral questionnaire underestimated the intake of middle-school children compared to a multiple-pass 24-hour recall [411]. Generally, correlations between food frequency type instruments and more precise reference instruments have been lower in child and adolescent populations than in adult populations. For these reasons, the food frequency approach is not recommended for children and adolescents.

New technology has been incorporated into some dietary assessment approaches. Williamson et al. [412] developed and tested an observational method using digital photography in school cafeterias. The method consists of standardized photography of the food selected before the meal and the plate waste following the meal. Using reference portions of measured quantities of the foods, expert judgment is used to estimate the amount of each food consumed [413]. Technology-based methods, such as disposable cameras, mobile phones with cameras [414], and smart phones, are being developed for collecting records and may be particularly useful among adolescents, who prefer these methods to traditional methods [415]. Examples of these new methods are the Remote Food Photography Method [416] and Technology Assisted Dietary Assessment [417]. Generally, these methods require more development, and eventual large-scale evaluation.

In addition to performance considerations, the choice of which dietary assessment approach instrument to use in a given study may depend on the study objectives and study design factors, all of which will influence the appropriateness and feasibility of different approaches [418].

D Elderly

Measuring diets among the elderly can, but does not necessarily, present special challenges [419−422]. Both recall and food frequency techniques are inappropriate if memory or cognitive functioning is impaired. Similarly, self-administered tools may be inappropriate if physical disabilities such as poor vision are present. Interviewer administration is difficult when hearing problems are present [421]. Direct observation in institutional care facilities [419] or shelf inventories for elders who live at home can be useful. Even when cognitive integrity is not impaired, several factors can affect the assessment of diet among the elderly. Because of the frequency of chronic illness in this age group, it is more probable that special diets (e.g., low sodium, low fat) would have been recommended. Such recommendations could not only affect actual dietary intake but also bias reporting because individuals may report what they should eat rather than what they do eat. Alternatively, respondents on special diets may be more aware of their diets and may more accurately report them. When dentition is poor, the interviewer should probe regarding foods that are prepared or consumed in different ways. Relative to other age groups, the elderly are more apt to take multiple types of nutritional supplements [423−425], which present special problems

TABLE 1.2 Optimal Strategies for Special Populations

Special Population	Optimal Strategies
Respondents unable to self-report	Use best-informed surrogate
	Analyze effect of potential bias on study results
Ethnic populations	Use interviewers of same ethnic background
	Use nutrient composition database reflective of foods consumed
	For FFQs, use appropriate food list and nutrient composition database
Children	For young children, use caretakers in conjunction with child
	For older children and adolescents, blended instrument and other creative ways of engagement and motivation may work best
	For FFQs, use appropriate food list and portion size categories
Elderly	Assess any special considerations, including memory, special diets, dentition, use of supplements, etc., and adapt methods accordingly

in dietary assessment (see Chapter 2: Assessment of Dietary Supplement Use). Because of the concern of malnutrition among the elderly, specific instruments to detect risk of malnutrition [426], such as the Mini Nutritional Assessment [427] and the Mini Nutritional Assessment Short Form [428,429], the Geriatric Nutritional Risk Index [430–432], the Subjective Global Assessment [426,428], and the Scored Patient-Generated Subjective Global Assessment [433] have been developed. While all of these tools focus on the elderly, they vary by setting, purpose, and administration mode.

Some researchers have suggested that the short-term memory required for the 24-hour recall may be more difficult for the elderly, who are more adept at long-term memory [419]. However, interviewers conducting an FFQ among elderly respondents noted difficulty in maintaining interest and concentration, whereas these issues were not found during the more engaging 24-hour recall interview [420].

Validation studies using doubly labeled water and/or urinary biomarkers among the elderly are limited [42,434–436]. Generally, energy underreporting has been found to be positively related to elevated BMI and lower education, similar to younger populations. However, in the NIH-funded Health, Aging, and Body Composition Study cohort, Shahar et al. [436] found that a substantial portion of elderly reporters were undereaters, losing more than 2% of their weight over a year. The distinction between undereating and underreporting is particularly relevant in the elderly.

Adaptations of standard dietary assessment methods have been suggested and evaluated, including using memory strategies, notifying the respondent prior to the dietary interview [437], combining methods [438], conducting multiple interviews for long protocols [419], and adapting existing instruments [439]. Specific adaptations that have been made in elderly populations include use of household measures rather than pictures to portray portion size for sight-impaired respondents [420] and tailoring the food list and portion sizes to be characteristic of the elderly rather than all adults in FFQs and their related databases [440,441].

Some have suggested including measures of cognitive function within a study to aid interpretation of results, but one such study found no relationship between cognitive functioning score and the validity of an FFQ [442]. In another study those showing cognitive dysfunction were excluded, but this creates selection bias [443]. Another approach is to solicit surrogate information for those considered cognitively unfit [444]. Mobile and web-based methods may prove useful, but currently the acceptance, feasibility, and validity of such methods in the elderly are unknown [422].

The variability in functional status among the elderly suggests the need for a flexible approach in assessing dietary intake. Mixed mode design in survey research [445] has certain advantages with regard to enhancing coverage and decreasing nonresponse, but it may cause other biases [446].

Table 1.2 summarizes special considerations for specific populations.

V SELECTED ISSUES IN DIETARY ASSESSMENT METHODS

A Cognitive Testing Research Related to Dietary Assessment

Nearly all studies using dietary information about subjects rely on the subjects' own reports of their diets. Because such reports are based on complex cognitive processes, it is important to understand and take advantage of what is

known about how respondents remember dietary information and how that information is retrieved and reported to the investigator. The need for and importance of such considerations in the assessment of diet has been discussed by several investigators [302,376,447–449], and research using cognitive testing methods [10,90,123,197,215,253,267,448,450–454] and other qualitative research techniques [400,402,404,455–458] has been reported. A thorough description of cognitive interviewing methods is found in Willis [459,460].

Specific and generic memories of diet are distinctly different. Specific memory relies on particular memories about episodes of eating and drinking, whereas generic memory relies on general knowledge about typical diet. A 24-hour recall relies primarily on specific memory of all actual events in the very recent past, whereas an FFQ that directs a respondent to report the usual frequency of eating a food during the previous year relies primarily on generic memory. As the time between the behavior and the report increases, respondents may rely more on generic memory and less on specific memory [448].

Investigators can do several things to enhance retrieval and improve reporting of diet. Research indicates that the amount of dietary information retrieved from memory can be enhanced by the context in which the instrument is administered and by use of specific memory cues and probes. For example, for a 24-hour recall, foods that were not initially reported by the respondent can be recovered by interviewer probes. The effectiveness of these probes is well-established and is therefore part of the interviewing protocols for all standardized high-quality 24-hour recalls, including those administered in the NHANES. Probes can be useful in improving generic memory, too, when subjects are asked to report their usual diets from periods in the past [302,449]. Such probes can feature questions about past living situations and related eating habits.

The way in which questions are asked can affect responses. Certain characteristics of the interviewing situation may affect particular responses for foods viewed as "good" or "bad." For example, the presence of other family members during the dietary interview may increase bias due to social approval or social desirability traits [333,334], especially for certain items such as alcoholic beverages. An interview in a health setting, such as a clinic, may also increase social approval bias in reporting about foods that were previously proscribed or recommended in that setting. In all instances, interviewers should be trained to refrain from either positive or negative feedback and should repeatedly encourage subjects to accurately report all foods.

B Validation Studies

Validation studies yield information about how well the primary or main method used to collect dietary data is measuring what it is intended to measure. It is important and desirable that the main dietary assessment method be evaluated against a less-biased reference method [179,180,182,461]. Furthermore, even if an instrument has been evaluated and shows satisfactory results, its proposed use in a different population may warrant additional validation research in that population. The purposes of such studies are to better understand how the method works in the particular research setting, to improve it if possible, and to use that information to better interpret results from the overall study.

There are two types of validation studies. The first assesses the validity of reported intakes for a specific number of days or meals in comparison to reference measures that approximate truth such as direct observation, feeding studies, or recovery biomarkers for a time period exactly consistent with each self-reported intake day. The results of this type of study provide estimates of differences in true versus reported intakes of nutrients and food groups, proportion of foods and drinks accurately reported and omitted, and correlation coefficients. This type of study can only be used for short-term instruments such as 24-hour recalls or food records. For example, if the 24-hour recall or food record is the main instrument in a study, available reference instruments include observational techniques, feeding studies, or recovery biomarkers [115,390,462,463]. In observation or feeding studies, accuracy can be assessed by determining the matches, intrusions and exclusions in the foods reported compared to true intakes, and for matches differences between actual and reported nutrient and food group intakes and portion sizes [93,464,465]. Recovery biomarkers are unbiased reference instruments and include 24-hour urine collections to measure protein, sodium, and potassium intakes and doubly labeled water which measures energy expenditure and is used as a measure of energy intake when individuals are in energy balance [41–47,98,167,168,170,171,466,467]. In studies using recovery biomarkers as the reference instruments, intakes estimated from the biomarkers can be compared to reported intakes from recalls or food records to assess reporting error. However, the high cost and increased respondent burden can make the collection of recovery biomarkers impractical for many studies. Additionally, known recovery biomarkers are limited in number.

The second type of validation study assesses how well reported intakes match true usual intakes and collects reference measures such as recovery biomarkers or less-biased self-report dietary assessment instruments for a time period not exactly consistent with each self-reported intake day. This type of validation study can be used across all self-report dietary assessment instruments when interest is in obtaining validation measures of usual intake. For example, when an FFQ is used as the main

study instrument, it can be evaluated in a study that compares it to another less-biased dietary assessment method, such as 24-hour recalls or dietary records and, preferably, to recovery biomarkers. The results are summarized by statistics such as correlation coefficients, bias, and attenuation factors. Correlation coefficients are related to the loss of power to detect relationships between diet and health outcomes. They are also useful for estimating the sample size required in a study because the less precise the diet measure, the more individuals will be needed to attain the desired statistical power [468]. Bias provides information about the difference between average reported intake and average true intake, at the group level. Attenuation factors represent bias in the estimated effect of self-reported dietary components on a health outcome. Some of this "attenuation bias," can be addressed through the use of measurement error models that allow for within-person error in the reference instrument, resulting in estimates that more nearly reflect the correlation between the diet measure and true diet [325,468]. It is important to note that when an FFQ is being evaluated using other biased and imperfect self-report reference instruments such as dietary records or 24-hour recalls, reporting errors between an FFQ and records/recalls are correlated, therefore, the statistical measures that result, such as correlation, bias, and attenuation, will be overly optimistic compared to those determined from unbiased reference instruments such as recovery biomarkers.

Validation and calibration studies (see below) are challenging because of the difficulty and expense in collecting reference dietary information. Because of this, such studies are done frequently on subsamples of the total study sample. If possible, the subsample should be chosen randomly. In addition, it should be sufficiently large to estimate the relationship between the study instrument and a reference method with reasonable precision. Increasing the numbers of individuals sampled and decreasing the number of repeat measures per individual (e.g., for an FFQ validation, collecting two nonconsecutive 24-hour recalls on 100 people rather than four recalls on 50 people) often can help to increase precision without extra cost [469]. The subsequent analyses quantify the relationship between the primary or main dietary intake tool and the reference method, and the resulting statistics can be used for a variety of purposes.

Too often, the term "validated" is used indiscriminately in research publications, to imply that the instrument is "valid," rather than that the instrument has been evaluated [470]. Thus the existence of a validation study is used by some to imply that the instrument is valid, regardless of the validation study's results. Often, validation coefficients in the range of 0.4−0.6 are presented as evidence that an instrument is valid. In reality, however, such findings should not be used to answer a "yes" or "no" question with respect to whether or not an instrument is "valid." Instead, readers should consider how the instrument performed for the purpose of study planning or instrument improvement. One should also consider whether the validation study design used unbiased or imperfect reference measures to evaluate the main instrument. The identification of additional unbiased references is needed to allow more extensive evaluation of self-report dietary assessment instruments.

The NCI maintains a register of validation/calibration studies and publications on the web [166].

C Calibration and Regression Calibration

The term "calibration" is used to refer to the rescaling of dietary data obtained from a more biased, less accurate instrument using information obtained from a less-biased, more accurate instrument. A calibrated instrument can be used to estimate population means and compare subpopulation means more accurately than an instrument that has not been calibrated. Calibration is distinct from "regression calibration," a term used to describe a method that uses calibration as part of a statistical procedure to better estimate associations (e.g., relative risks) between diet and other factors, such as health outcomes.

Calibration can be used to relate reported intakes on an FFQ or screener to a more accurate reference instrument administered in the same population. For example, a study may administer an FFQ to all respondents and the reference instrument (such as 24-hour dietary recalls) to a subsample. Alternatively, external calibration using data from a reference population different from the study population can be performed. In this case, the external population should be similar to the study population. In both situations, scoring algorithms are estimated and used to rescale the dietary data from the screener. The use of such scoring algorithms for screeners has been shown to lead to estimates of mean intakes that are closer to means estimated with 24-hour recall than those derived solely from screeners.

Regression calibration is a method used to adjust estimates of associations between diet and health outcomes for measurement error. This requires a main dietary assessment instrument collected among all study subjects and a reference instrument collected in at least a subsample. This data to accomplish regression calibration often come from a validation study (described above). In cohort studies, the main instrument has most often been an FFQ, although the use of multiple recalls or multiple-day food records is now more feasible than in the past. The estimated regression relationship between an FFQ and the reference method is used to adjust the relationships between diet and outcome (e.g., relative risk of disease for subjects with high nutrient intake compared to those

with low intake) as assessed in the larger study [164,175,176,325,471,472]. Many of these adjustments require the assumption that the reference method is unbiased [175,323]. However, as discussed above, at least for most nutrients and food groups, the reported intakes from reference instruments such as recalls and records are biased in a manner correlated with FFQ [149], violating this assumption, which leads to overestimates of validity. For these reasons, researchers use recovery biomarkers such as urinary nitrogen and doubly labeled water when possible because they are unbiased measures of intake. However, because these are available for only a few nutrients, data from imperfect reference instruments such as 24-hour dietary recalls or food records are used. Such data are assumed to be unbiased for true usual intake, even though they fall short of this ideal. Although using these imperfect reference instruments does not completely adjust estimated diet-outcome associations for the bias caused by dietary measurement error, on average, it may produce less-biased results than an unadjusted standard analysis based solely on FFQ data. Another area in need of further study is the effect of measurement error in a multivariate context because most research thus far has been limited to the effect on univariate relationships [178,182,473,474].

D Mode of Administration

Instruments may be interviewer-administered or self-administered. Interviewer-administered questionnaires may be in person or by telephone. A self-administered instrument may be completed on paper or electronically. All of these modes are currently used for dietary assessment.

For interviewer-administered instruments, telephone administration is less costly than in-person administration. However, concern is increasing about response rates in telephone surveys, given the public's distaste for prevalent telemarketing, technology that allows for screening of calls, the increase in the proportion of the population (especially young adults [475]) who use only wireless telephones, and the general resistance of the public to engage in telephone interviews. For these reasons, response rates obtained using random digit dialing techniques have been dropping.

Despite these difficulties, many surveys and studies do collect dietary data over the telephone. For example, BRFSS [193] and the CHIS [195], both, include dietary screeners. NHANES [299] administers an initial 24-hour recall at the examination site and a second 24-hour recall later by telephone. For 24-hour recalls collected by telephone, the difficulty of reporting serving sizes can be eased by mailing picture booklets or other portion size estimation aids to participants before the interview. Many studies have evaluated the comparability of data from telephone versus in-person 24-hour recall interviews. Several have found substantial but imperfect agreement between dietary data collected by telephone and that estimated by other methods, including face-to-face interviews [74,476–478] or observed intakes [479]. Godwin et al. [480] and Yanek et al. [481] examined the accuracy of portion size estimates for known quantities of foods consumed that were assessed by telephone and by in-person interviews. Both estimates were found to be similarly accurate.

Self-administration is less costly than interviewer-administration. In addition, self-administered surveys tend to minimize social desirability bias [482]. However, self-administration may not be feasible for segments of the population who have low literacy levels or limited motivation. Thus, selection bias is a potential problem.

Web-administered questionnaires have cost advantages and have become popular as the penetrance of the Internet increases. In 2013, 79% of households in the United States had Internet access [483]. Various FFQs [122], dietary history questionnaires [484], screeners [250,485], and 24-hour recall instruments [76,88,486] have been developed for web administration. In general, it has been found that initial response rates for web questionnaires are substantially lower than those for mailed or telephone interviewer questionnaires [487]. One study conducted in Sweden found a lower initial response rate to a web questionnaire compared to a mailed printed questionnaire but greater compliance in answering follow-up questions over the web [488]. Web-administered questionnaires may be more effective than telephone interviewer-administered questionnaires for presentation of complex questions that are better processed visually than aurally by respondents and that can be answered at a pace set by the respondent rather than by the interviewer [489]. Beasley et al. [490] found that the responses to questions about diet on a web-administered FFQ were not significantly different from responses on a paper version of the same questionnaire. One large-scale survey found that self-administered 24-hour recalls using the Internet yielded nutrient intake estimates similar to interviewer telephone-administered 24-hour recalls [94]. The Internet version was preferred over the telephone-administered version by 70% to 30% [94].

Dietary assessment with mobile phones or tablets is an active area of development and research. Several self-administered 24-hour recalls instruments are available on mobile devices [76]. Use of mobile phones to record and photograph foods is also possible [491,492]. Sharp et al. recently reviewed evaluative studies of mobile phones to assess diet [493] and found that validity was comparable but not superior to other conventional methods. Further studies in larger and more diverse populations comparing these mobile devices to other modes of data collection are

needed to examine comparability as well as the potential for self-selection biases.

E Estimation of Portion Size

Research has shown that untrained individuals have difficulty in estimating portion sizes of foods, both when examining displayed foods and when reporting about foods previously consumed [91,389,399,480,494–510]. One study indicates that literacy, but not numeracy, is an important factor in an individual's ability to accurately estimate portion size [511]. Furthermore, respondents appear to be relatively insensitive to changes made in portion size amounts shown in reference categories asked on FFQs [512]. Portion sizes of foods that are commonly bought and/or consumed in defined units (e.g., bread by the slice, pieces of fruit, and beverages in cans or bottles) may be more easily reported than amorphous foods (e.g., steak, lettuce, and pasta) or poured liquids [91,509]. Other studies indicate that small portion sizes tend to be overestimated and large portion sizes underestimated [496,508,513].

Aids are commonly used to help respondents estimate portion size. Research showing that different types of aids are more or less effective for different types of foods [417,510,514] indicates that having multiple types of aids available may be optimal. The NHANES What We Eat in America uses an extensive set of three-dimensional models for an initial in-person 24-hour dietary recall [515]. Respondents then are given a Food Model Booklet developed by the USDA [516] along with a limited number of three-dimensional models and household measures (e.g., measuring cups and spoons) for recalls collected by telephone. Food pictures and models have been developed for other eating patterns, for example, Asian foods [517] and foods consumed in Mexico [518]. The accuracy of reporting using either models or household measures can be improved with training [412,519–521], but the effects may deteriorate with time [522]. Studies comparing the use of either household measures or pictures among children and adolescents indicate that pictures outperform household measures [514,518]. Studies that have compared three-dimensional food models to two-dimensional photographs in adults have shown that there is little difference in the reporting accuracy between methods [388,480,523,524]. One study in children, however, showed that using food models resulted in somewhat larger error than using digital images [506]. Portion size pictures, however presented, should be tailored to the particular populations and ages.

With the increased use of technology in dietary assessment, digital food images in multiple portion sizes are being tested. Studies have investigated the effects of number of portion pictures, size of picture, and concurrent versus sequential display on accuracy of report [91,399,505]. Such studies indicate preferences by respondents but generally little difference in accuracy. However, in two studies, one with adults [91] and the other with children [400], accuracy was higher when more portion size choices were offered. An emerging use of digital technology removes respondent judgments of portion size, instead relying on digital images of foods taken before and after consumption, either actively by the respondent [525,526] or passively by a wearable camera [527,528]. Computer software is then used to both identify foods and estimate the amount consumed.

F Choice of Nutrient and Food Database

It is necessary to use a nutrient composition database when dietary data are to be converted to nutrient intake data. Typically, such a database includes the description of the food, a food code, and the nutrient composition per 100 g of the food. The number of foods and nutrients included varies with the database. Research on nutrients, other dietary components, and foods is ongoing, and there is constant interest in updating current values and providing new values for a variety of dietary components of interest.

Some values in nutrient databases are obtained from laboratory analysis; however, because of the high cost of laboratory analyses, many values are estimated based on conversion factors or other knowledge about the food [529]. In addition, accepted analytical methods are not yet available for some nutrients of interest [530], analytical quality of the information varies with nutrient [530,531], and the variances or ranges of nutrient composition of individual foods are in most cases unknown but are known to be large for some nutrients [532]. Rapid growth in the food processing sector and the global nature of the food supply add further challenges to estimating the mean and variability in the nutrient composition of foods eaten in a specific locale.

One of the USDA's primary missions is to provide nutrient composition data for foods in the U.S. food supply, accounting for various types of preparation [533]. Information about the USDA's nutrient composition databases is available at the USDA's Nutrient Data Laboratory home page [534]. The USDA produces and maintains the Nutrient Database for SR. New releases are issued yearly; these include information on new foods and revised information on already included foods, and they identify foods deleted from the previous version of the database. The most recent release, SR28, includes information on up to 150 food components for 8789 foods [535], and is available online.

Interest in nutrients and food components potentially associated with diseases has led the USDA to develop specialized databases for a smaller number of food

components, such as flavonoids [534]. A separate database developed by the USDA Food Surveys Research Group—the Food and Nutrient Database for Dietary Studies (FNDDS)—is used by many investigators in analyses of foods reported in NHANES' What We Eat in America dietary recalls and is based on nutrient values in the USDA SR database [92]. The FNDDS provides information for 65 nutrients and food components, and has no missing data for nutrient fields.

Nutrient composition data are also compiled by a number of other countries, and the International Network of Food Data Systems maintains an international directory of nutrient composition tables [536]. Combining different food composition databases across countries poses comparability challenges, however. The European Food Information Resource [537] was formed to support the harmonization of food composition data among the European nations [538]. The International Nutrient Databank Directory, an online compendium developed by the National Nutrient Databank Conference, provides information about the data included in a variety of databases, national reference databases, and specialized databases developed for software applications, such as the date the database was most recently updated, the number of nutrients provided for each food, and the completeness of the nutrient data for all foods listed [539].

In addition to nutrient databases, databases that relate dietary intake to dietary guidance have been developed in the United States [540,541]. The USDA Food Patterns Equivalents Database (FPED) provides quantities of specific food groups consistent with dietary guidance recommendations in order to allow for evaluation of whether diets meet dietary guidelines at a variety of calorie levels [542]. Just as FNDDS provides nutrient composition data, the FPED provides food group data per 100 g of each food code in FNDDS. Importantly, mixed dishes, such as pizza, are disaggregated to their food group components. The FPED contains data for 37 food group components (e.g., dairy, fruits, vegetables) [543].

Other databases are available in the United States for use in analyzing dietary records and 24-hour recalls, but most are based fundamentally on the USDA SR database, often with added foods and specific brand names. One prominent such database is the University of Minnesota's Nutrition Coordinating Center's (NCC) Food and Nutrient Database [544]. This database includes information on 165 nutrients, nutrient ratios, and other food components for more than 18,000 foods, including 8000 brand-name products. The NCC is constantly updating its database to reflect values in the latest release of the USDA SR database.

One limitation in all nutrient databases is the variability in the nutrient content of foods within a food category and the volatility of nutrient composition in manufactured foods. Recent changes in the sodium and fatty acid composition of manufactured foods, for example, illustrate the difficulty in maintaining accurate nutrient composition databases [545,546]. Obviously, a key consideration is how the database is maintained and supported.

Estimates of nutrient intake from 24-hour recalls and dietary records are often affected by the nutrient composition database that is used to process the data [547–549]. Inherent differences in the database used for analysis include factors such as the number of food items included in the database, how recently nutrient data were updated, and the number of missing or imputed nutrient composition values. Therefore, before choosing a nutrient composition database, a prime factor to consider is the completeness and accuracy of the data for the nutrients of interest. For some purposes, it may be useful to choose a database in which each nutrient value for each food also contains a code for the quality of the data (e.g., analytical value, calculated value, imputed value, or missing). Investigators need to be aware that a value of zero is assigned to missing values in some databases, whereas for other databases, the number of nutrients provided for each food may fluctuate depending on whether or not a value is missing, and for others all unknown values may be imputed.

The nutrient database should also include weight/volume equivalency information for each food item. Many foods are reported in volumetric measures (e.g., 1 cup) and must be converted to weight in grams in order to apply nutrient values. The number of common mixtures (e.g., spaghetti with sauce) available in the database is another important factor. If the study requires precision of nutrient estimates, then procedures for calculating the nutrients in various mixtures must be developed and incorporated into nutrient composition calculations.

Developing a nutrient database for an FFQ presents additional challenges [550] because each item on the FFQ represents a food grouping rather than an individual food item. Various approaches that rely on 24-hour recall data, either from a national population sample or from a sample similar to the target population, have been used [551–553]. Generally, individual foods reported on 24-hour recalls are grouped into FFQ food groupings, and a composite nutrient profile for each food grouping is estimated based on the individual foods' relative consumption in the population. For this approach to be effective, the 24-hour recall data must be representative of the population for whom the FFQ is designed and connected to a trustworthy nutrient database.

G Choice of Dietary Analysis Software

Data processing of 24-hour recalls and dietary record requires creating data that include a food code and an

amount consumed for each food reported. Computer software then links the nutrient composition of each food on the separate nutrient composition database file, converts the amount reported to multiples of 100 g, multiplies by that factor, stores that information, and sums across all foods for each nutrient for each individual for each day of intake. Many software packages have been developed that include both a nutrient composition database and software to convert individual responses to specific foods and, ultimately, to nutrients. A listing of many commercial dietary analysis software products has been compiled [539].

Software should be chosen on the basis of the research needs, the level of detail necessary, the quality of the nutrient composition database, and the hardware and software requirements [554]. If precise nutrient information is required, it is important that the system be able to expand to incorporate information about newer foods in the marketplace and to integrate detailed information about food preparation by processing recipe information (e.g., the ingredients and cooking steps for homemade stew). Sometimes the study purpose requires analysis of dietary data to derive intake estimates not only for nutrients but also for food groups (e.g., fruits and vegetables), food components other than standard nutrients (e.g., nitrites), or food characteristics (e.g., fried foods). These additional requirements limit the choice of appropriate software.

The semiautomated food coding system used for NHANES is USDA's Dietary Intake System, consisting of the AMPM for collecting food intakes; the Post-Interview Processing System, which translates the AMPM data and provides initial food coding; and the Survey Net food coding system for the final coding of the intake data [86]. Survey Net is a network dietary coding system that provides online coding, recipe modification and development, data editing and management, and nutrient analysis of dietary data; multiple users can use the software to manage the survey activities. It is available to government agencies and the general public only through special arrangement with the USDA. NCI's ASA24 instrument performs automated coding of all reported foods. Foods which are not completely described are assigned default values.

Many diet history and food frequency instruments have also been automated. Users of these software packages should be aware of the source of information in the nutrient database and the assumptions about the nutrient content of each food item listed in the questionnaire.

H Estimating Usual Intakes of Nutrients and Foods

Usual intake is conceptualized as the long-term average intake of a food or nutrient. The concept of long-term average daily intake, or "usual intake," is important because dietary recommendations are intended to be met over time and diet–health hypotheses are based on dietary intakes over the long term. Consequently, it is the usual intake that is often of most interest to policymakers (e.g., the proportion of the population at or below a certain level of intake) or to researchers (e.g., relationships between diet and health).

Data from FFQs, 24-hour recalls, and dietary records have all been used to estimate usual intake at the group level. Obtaining accurate estimates of usual intake at the individual level is generally not possible with the dietary assessment tools available even for FFQs which attempt to estimate usual intake generally over a longer period such as the past year. FFQs are known to contain a substantial amount of measurement error (see Section II.C) [54,79,100–103,117,149]. Dietary recalls or records generally provide more accurate short-term intake estimates than frequency-type instruments.

For estimates of mean usual intake in the population, data from just a single day of recall or record can be used. Multiple days of recalls and records are needed to estimate the distribution of intakes. However, the distribution of simple within-person averages of intakes across a few days does not adequately represent the population's usual intake distribution [555], because of the large day-to-day variability of individuals' diets. Distributions generated from averaging only a few days of data are generally substantially wider than those of true usual intakes, and thus lead to overestimating the proportion of the population above or below a certain cut point, as illustrated in Fig. 1.1.

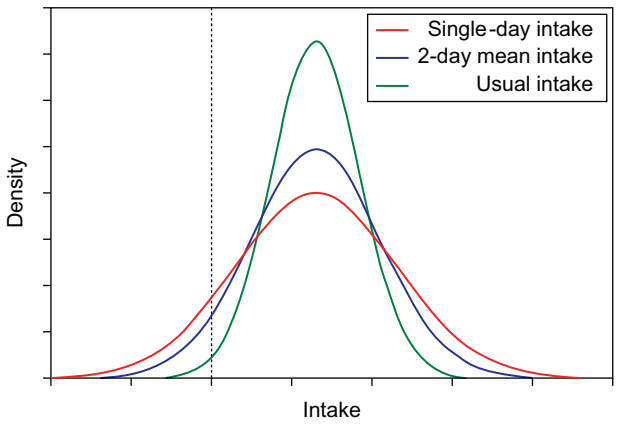

FIGURE 1.1 Effect of day-to-day variability on distributions. *Adapted from NCI Dietary Assessment Primer, Epidemiology and Genomics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute. Available from https://dietassessmentprimer.cancer.gov/.*

Statistical modeling can be used to more accurately portray the population's distribution by analytically estimating and removing the effects of day-to-day variation in dietary intake [555]. These methods rely on a minimum of two administrations of 24-hour recalls or dietary records to capture day-to-day variation. The earliest efforts at statistical modeling of usual intake were made by the Institute of Medicine [556] for nutrients, most of which are consumed nearly every day by most everyone, and then extended and updated for nutrients or foods that are more episodically consumed (e.g., dark green vegetables) by researchers at Iowa State University [557–559]. Others have developed usual intake statistical approaches as well [189,560–563]. The NCI method uses a minimum of two 24-hour recalls to estimate intake of both nutrients and episodically consumed foods [296]. This model as well as others [189] allows for covariates such as sex, age, race/ethnicity, or information from an FFQ to supplement the model [562]. One study using the NCI method showed that including FFQ data as covariates in modeling usual intakes from 24-hour recalls increased precision for assessing the relationship of a highly episodically consumed food, fish, with blood mercury levels [190]. Modeling usual intakes to assess relationships to health outcomes by combining data from a few 24-hour recalls with an FFQ has been shown to provide better estimates compared to a single FFQ or a few 24-hour recalls alone [188,189,295].

The NCI Measurement Error Webinar Series [564] provides a thorough discussion of dietary measurement error, including usual intake estimation.

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Chapter 2

Assessment of Dietary Supplement Use

Johanna T. Dwyer, Rebecca B. Costello and Joyce Merkel
National Institutes of Health, Bethesda, MD, United States

I INTRODUCTION

A Rationale

Nutritional status assessment is incomplete without assessing both the intake of food and dietary supplements because supplements provide many essential nutrients and other bioactive substances that may affect health outcomes in both beneficial and harmful ways. For those suffering from health problems, specific dietary supplements may be recommended or self-prescribed. Moreover, the prevalence of supplement use is high [1,2].

Dietary supplements are marketed in a variety of formulations, including drinks, bars, tablets, pills, and powders. For the purpose of discussion in this chapter, they are defined using the legal definitions in the Dietary Supplement Health and Education Act (DSHEA) of 1994: "a product (other than tobacco) that is *intended to supplement the diet; contains one or more dietary ingredients* (including vitamins, minerals, herbs, or other botanicals, amino acids, and other substances) or their constituents; is *intended to be taken by mouth* as a pill, capsule, tablet, or liquid; and is *labeled on the front panel as being a dietary supplement*" [3]. Note that in the United States herbals, botanicals, and other nonvitamin, nonmineral products are included in the definition of dietary supplements; in many other countries they are categorized as medicines.

B Purposes of Dietary Supplement Intake Assessment

1 Obtain Total Intakes of Nutrients

Dietary supplements contribute substantial amounts to total intakes of some nutrients, such as calcium and vitamin D in postmenopausal women, and in "renal vitamins" (folic acid, vitamin B_6, and vitamin B_{12}) provided to hemodialysis patients to replace losses of water-soluble vitamins during dialysis. Failure to include these nutrient sources would lead to serious underestimation of intakes and overall diet quality. This is important since more than half of American adults use dietary supplements, and nearly half of them use multivitamin−mineral (MVM) supplements. Most of the rest use single or multiple vitamin or mineral preparations that also contribute substantial amounts of nutrients to dietary intakes [2]. Therefore, it is essential to assess total intakes, including dietary supplements among users of these products.

2 Assess Risk of Toxicities

Supplements are often highly concentrated sources of nutrients, especially when use of highly fortified foods is high. Some individuals may have nutrient intakes that are so excessive that tolerable upper levels of nutrients may be exceeded, placing the individual at risk of toxicity. Some of the bioactives in supplements can also have toxic effects, as was the case with the botanical ephedra, which was used in many weight-loss and performance-enhancing dietary supplements until banned by the Food and Drug Administration (FDA) in 2004 [4]. For example, in 2012 in the United Kingdom, 84 illegal products, such as energy and muscle gain products, were found to contain dangerous ingredients including steroids, stimulants, and hormones [5]. Some dietary supplements may be spiked with active drugs not declared on the label; others are adulterated with other substances or contaminated by heavy metals, pesticides, filth, or other toxic ingredients—another reason for noting supplement use on medical records. Finally, dietary supplements include herbals and botanicals that are sometimes used as medicines by those who are ill, and who may be taking other medications as well that may interact with them to produce adverse effects. Although the DSHEA prohibits the use of supplements for the prevention, mitigation, or cure of disease, these uses appear to persist, increasing the risk of toxicities.

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3 Assess Supplement–Drug and Supplement–Nutrient Interactions

These interactions are important to document in order to avoid drug or nutrient–supplement interactions. Supplement–nutrient and supplement–drug interactions are of particular concern. Some dietary supplements, particularly botanicals, contain many bioactive substances that may interact with other drugs or nutrients [6]. The Centers for Disease Control and Prevention (CDC) estimated that 23,000 emergency department visits in the United States every year were due to adverse effects related to dietary supplements of which 2154 resulted in hospitalizations [7]. Many older persons are already taking multiple medications, and they are at risk of polypharmacy even without use of supplements. There is evidence that the use of prescription medications and dietary supplements often increases the risk of interacting medications, and that a substantial and growing proportion of older adults are at risk for major drug–drug interactions [8].

4 Clarify Associations Between Dietary Supplement Intake, Healthcare, and Health Status

It is important to clarify associations between dietary supplement use and health, including risk of various diseases and changes in supplement use with changes in health status. The associations between use of supplements and both conventional and alternative medicines or other lifestyle factors may also be revealing [9,10].

5 Assess Conformity with Health Promotion and Disease Prevention Recommendations and Guidelines

The Food and Nutrition Board (FNB) of the Division of Medicine, National Academy of Medicine, the U.S. Preventive Health Services Task Force, Healthy People 2020, the Committee on Nutrition of the American Academy of Pediatrics, and consensus statements issued on behalf of these and other professional societies make recommendations on the use of dietary supplements. Health screening tools may include queries to ascertain if patients are following these guidelines. For example, the FNB recommends that women in the child-bearing years who are at risk of becoming pregnant should take a dietary supplement of folic acid and ensure that their food intake of folate is adequate.

C Dietary Supplements Available

It is estimated that over 7000 entities are involved in the manufacturing, marketing, and distribution of dietary supplements. The number of supplement products is very large, with estimates ranging to as high as 80,000, with a turnover as high as 30% per year. There are at least 5600 manufacturers, packagers, and suppliers of supplements in the United States, and there are many more concerns abroad that provide the ingredients in supplements. These operations run the gamut from very sophisticated facilities with high pharmaceutical standards and quality controls to very poor facilities. This is important since both the safety and efficacy of supplements depend on high quality ingredients and products. The Office of Dietary Supplements (ODS) at the National Institutes of Health's (NIH's) web-based Dietary Supplement Label Database (DSLD) contains 60,000 labels, and continues to add 1000 labels per month. The DSLD provides images of each product and all information on the label. This resource can be accessed at https://www.dsld.nlm.nih.gov.

D Health Profiles of Dietary Supplement Users

The health profiles of dietary supplement users differ from those of nonusers in important respects that affect health, making causative associations between supplement use and health status difficult to establish without first correcting for supplement use. These factors include physical activity, smoking status, age, income, education, and prior health status. For example, MVM supplement users tend to have better diets and to be healthier than nonusers, probably because of the differential presence of factors mentioned previously. However, generalization is hazardous, and those who are ill often use supplements as well. In a prospective cohort study of 25,874 overweight older adults with multiple risk factors for chronic diseases, 44.8% were consuming multivitamins, 42.6% consumed $<$800 IU/day of vitamin D, and 26.4% consumed $<$1200 mg/day of calcium [11]. Cancer survivors often have very high rates of dietary supplement use in the hope that the use of supplements will stave off a return of the malignancy, and those with advanced stages of cancer often have even higher rates of use [12]. Users of "condition-specific" supplements often suffer from specific health problems. There are many illnesses that consumers believe will be alleviated or cured by dietary supplement use. For example, glucosamine and chondroitin or chondroitin sulfate are often used as medicines by those who are already ill or suffering from joint pain or osteoarthritis [13]. Saw palmetto is commonly used by those with prostate problems or by prostate cancer survivors [14,15]. Use of dietary supplements in pregnant women at risk of developing diabetes is also exceedingly high—92% of women enrolled in The Environmental Determinants of Diabetes in the Young (TEDDY) study used one or more supplements during pregnancy [16].

E Prevalence of Dietary Supplement Use

The prevalence of dietary supplement use increased when supplements became more widely available after the passage of DSHEA in 1994. Today, prevalence is high, with more than half of all adults using some dietary supplements. There are substantial, but somewhat lesser, numbers of children using dietary supplements. The best data on prevalence of use are from population-based samples, such as the National Health and Nutrition Examination Survey (NHANES). On the basis of such studies, it is evident that use is particularly high in certain subgroups. For example, it is very high among elders, somewhat less but also high among young children, and lower in adolescents and adults [17,18]. Supplement use is also often positively associated with educational status, income, and, in most cases, better health status. However, those whose current health status is poor are often also heavy users of particular supplements in the hope that the products will mitigate or cure their conditions. Other studies reveal that some individuals, such as those on hemodialysis for end-stage renal disease; those postbariatric surgery with a form of malabsorption; and old, frail institutionalized patients, often take special supplements.

Limited surveys of dietary supplement use have also been done for health professionals such as dietitians, but the response rates in existing surveys are too low to provide data that can be extrapolated to the profession as a whole [19].

The *Nutrition Business Journal* publishes a list of the most popular dietary supplements each year, and the latest data are summarized in Table 2.1. Although the sales data on which these lists are based combine sales volume and the price of the product, and so they do not conform precisely to dietary supplement use. However, they do give some idea of what dietary supplements Americans are buying today. Data in *Nutrition Business Journal 2014* show that many herbal and botanical products, including most "super fruit" products and category leaders such as green tea and ginkgo biloba, decreased in sales compared to earlier years, whereas sales of magnesium, probiotics, and melatonin experienced double-digit increases from prior years [20].

The dietary supplement marketplace is constantly changing, and as it does, consumption patterns also change, so it is important to keep up with industry trends [21]. According to proprietary data collected by the Natural Marketing Institute using Harris Interactive survey data of U.S. adults (18 + years) from 2005 to 2015, use of "condition-specific supplements" (used for specific health issues such as memory, weight loss, and bone and joint health) has remained stable, 44% in 2005 and 44% in 2015. Use of herbal supplements also remained relatively stable with 46% of U.S. adults using in 2005 and 48% using today. Use of single minerals and vitamins exhibited some minor declines, both declining approximately 6% since 2005. In addition, other supplements, such as omega-3 fatty acids and probiotics, continue to drive growth in the dietary supplement category [22]. A 2010 Ipsos-Reid survey showed that 73% of Canadians regularly take natural health products such as vitamins and minerals, herbal products, and homeopathic medicines [23].

The FDA completed a national telephone survey of 2480 adults in the continental United States in 2014 [24]. Over the previous year, 60% of adults reported using multivitamin/multimineral or single ingredient vitamins or minerals, and 32% reported using herbal and nonvitamin/mineral supplements. Up to 83% of the vitamin mineral users looked for product information before using a product for the first time, and they got most of their information from product labels or traditional healthcare professionals. Among the herbal users, 93% got product information before using a product for the first time from the label, but they reported getting information mostly from product labels, family and friends, and the Internet. While 60% of the vitamin mineral users thought the government set standards or preapproved these products before they were sold, only 40% believed this for herbal products.

F Patterns of Dietary Supplement Use Among the Ill

Nutrition professionals need to be aware that people use dietary supplements in various ways when they fall ill, and that they are not always forthcoming about what they are doing when discussing their health problems with their physicians or other health professionals. In part, this is due to many physicians not asking their patients about supplement use. Also, patients sometimes assume that it is not important to mention supplement use, or they lack candor because they are afraid that the doctor will disapprove of use of the supplement.

Dietary supplements sold over the counter are not viewed as medications. DSHEA classified dietary supplements as foods, for which there are less stringent standards for quality and efficacy than would be true if they were categorized as drugs. By definition, under the DSHEA law, dietary supplements are designed to supplement the diet. They are not to be used for the prevention, mitigation, or cure of disease, although in fact many people use them for these purposes [25].

The vast majority of the American public turns first to prescription drugs if they are ill. A smaller proportion of the public turns first to dietary supplements or other alternative medicines for treating their illnesses, and as a

TABLE 2.1 Most Popular Dietary Supplements Based on 2013 Sales as Reported in *Nutrition Business Journal 2014*

Product	Consumer Sales ($ Millions)
Multivitamins	5712
Sports nutrition powders/formulas	3867
B vitamins	1784
Vitamin K, H, Other	1421[a]
Probiotics	1191[b]
Fish/animal oils	1168
Homeopathies	1138
Vitamin C	1044
Glucosamine/chondroitin	780
Vitamin D	713
CoQ10	607
Magnesium	583[a]
Sports nutrition drinks	437[a]
Vitamin A/β carotene	411
Iron	344
Melatonin	323[b]
Vitamin E	320
Plant oils	309
Other specialty	273
Digestive enzymes	243
Noni juice	225
Sports nutrition pills	213
Mangosteen juice	173
Saw palmetto	145
Cranberry	141[a]
Echinacea	139[a]
Turmeric	135[b]
Bee products	129[a]
Green tea	129[c]

[a]*Increased sales by 10% from previous year.*
[b]*Increased sales by 20% from previous year.*
[c]*Decreased sales by 10% from previous year.*
Source: As reported in *Nutrition Business Journal*, *Nutrition Business Journal Supplement Business Report*, New Hope Natural Medicines, Penton Media, Inc., San Diego, CA, 2014.

result they may delay obtaining care from licensed medical practitioners. Despite the limitations of the legitimate uses of dietary supplements solely to supplement the diet under DSHEA, data presented above would support that dietary supplements are often used in ways that are different than the law intends. Caution is indicated, especially for those who are undergoing medical treatment or who are ill. Dietary supplements are inappropriate as substitutes for evidence-based medical therapies prescribed by physicians and should not be used by the ill

without checking with a knowledgeable physician since they are not manufactured as pharmaceutical grade products. In most cases, the effectiveness of dietary supplements has not been demonstrated, and the potential for supplement–drug interactions also exists. Some consumers use a combination of prescribed drugs, over-the-counter drugs, and dietary supplements which they take simultaneously. Those who are ill run the highest risks of potential supplement–drug interactions, particularly if they are taking many medications and if they are taking medications or supplements that are especially likely to interact adversely with each other. For example, those on coumadin, a commonly used anticoagulant, may experience adverse reactions if they self-medicate with various herbal and botanical drugs that affect blood clotting [26,27].

Many consumers still view dietary supplements as harmless and possibly even helpful in the prevention and treatment of many conditions, including arthritis, colds and flu, osteoporosis, lack of energy, memory problems, and cancer, and hence they often self-medicate for these purposes. Individuals may also think that supplements have a role in the prevention or treatment of depression, stress, heartburn, high cholesterol, vision, and heart and blood pressure problems. Indeed this may not be the case. Vitamin mineral supplements are used more commonly than herbals and botanicals and other nonvitamin, nonmineral supplements for overall health and wellness, and for prevention and treatment, although their efficacy in many of these regards is unproven.

The FDA periodically issues health advisories on dietary supplements that pose special health risks. The alerts are at http://www.fda.gov/safety/recalls/default.htm. Websites that are helpful in assessing possible interactions of dietary supplements with drugs include the National Library of Medicine (NLM) MedLine Plus at https://www.nlm.nih.gov/medlineplus/druginformation.html, a portal for reliable health information in English and Spanish easily accessible in a variety of formats, including a mobile app, widgets, and buttons. The National Center for Complementary and Integrative Health (NCCIH) website at http://www.nccih.nih.gov, the Natural Medicines Database, https://naturalmedicines.therapeuticresearch.com/ (subscription database), Consumer Lab at http://www.Consumerlab.com, and the Center for Education and Research on Therapeutics at NCCIH suggest that physicians and patients discuss together the use of dietary supplements to maximize benefits and minimize risks. "Time to talk" materials to facilitate professional–patient dialogue on use of supplements are available at the NCCIH website at http://nccih.nih.gov/health/providers. Some disease focused groups such as the National Kidney foundation provide disease-specific advice to patients, such as lists of herbs that kidney patients should avoid, which can be accessed at https://www.kidney.org/atoz/content/herbalsupp.

Populations with poor reported physical or emotional health status tend to be users of herbal, botanical, condition-specific, and other types of supplements. Therefore, it is vitally important to check dietary supplement use in the ill. If patients are undergoing medical treatment and prescription drugs have been prescribed, they should be encouraged to use them as directed first and by themselves to gain the full effects of the therapy. If the patient insists on continuing dietary supplement use, possible adverse interactions with the drug regime should be investigated, and the patient should then be counseled on how to avoid potential adverse interactions. Any interactions identified should be entered into the patient's chart. If the interaction is severe or life threatening, it should be reported to the FDA at 1-800-FDA-1088 or http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053074.htm.

G Motivations for Dietary Supplement Use

Consumer confidence is high regarding how supplements can improve health and help manage many conditions [28]. People have many different motivations for dietary supplement use. Motivation varies not only from person to person and by such factors as demographics (age, sex, income, education, and ethnicity) but also by attitudes such as concerns about deficiencies, who prescribes it, readiness to engage in preventive behaviors, and health status. Motivation also varies over time within and between individuals, depending on the type of product, whether it is a nutrient or nonnutrient supplement, and the definition of dietary supplement that is employed.

Motivation and use of dietary supplements are related to each other but probably in complex ways that differ from one individual to another. One theory is that knowledge and attitudes (motivations) cause supplement use. It is also possible that people use supplements and then attitudes and knowledge (motivation) follow perhaps to rationalize or justify use. That is, some people apparently get into the habit and then find reasons for their behavior, often due to social influence, whereas others operate in a more deliberate manner, gathering knowledge and then forming attitudes that determine their use. The implications for nutrition and other health professionals of these different ways that people arrive at supplement use are that they must consider both ways to influence behavior when interviewing on dietary supplement use. For example, social influence may have utility in developing methods for persuading women in the reproductive age group who might become pregnant to increase their use of folic acid.

II METHODS FOR ASSESSING DIETARY SUPPLEMENT INTAKE

A Dietary Supplement Intake (Exposure)

Most of the methods for assessing intake of dietary supplements are similar to those discussed in Chapter 1, Dietary Assessment Methodology. The methods have the same strengths and limitations as those mentioned in that chapter. Another method that is applicable to supplements but not to foods is the use of pill inventories, which are widely used in obtaining information about other medications. These can be lists of commonly used supplements, or use the "brown bag" method; the healthcare provider asks the patient to bring in all their supplements to the office in a bag, or medication inventory "apps" can also be used. For some supplements, inferences about use can be made from blood or urine biomarkers, if available, although they provide only qualitative rather than quantitative information, and the tests are not available in most clinical settings. The unique features of the methods for collecting dietary supplement information are detailed in Table 2.2.

B Assessing Supplement Intake in Clinical Settings

1 Inpatient Settings

The chances of drug−drug, drug−supplement, and other adverse reactions are great among hospitalized patients. Most hospitals prohibit self-medication with dietary supplements or other over-the-counter medications without the written permission of the physician, and so supplement use in hospital inpatient settings is usually limited. However, prior use is of interest because some botanicals may take days or weeks to be excreted from the body, and it never hurts to ask about current use, since some patients disregard instructions. In the Joint Commission Comprehensive Accreditation Manual for Hospitals (JCAHO), a medication is defined as: "any prescription medication, sample medication, herbal remedies, vitamins, nutriceuticals, over the counter drugs, vaccines, diagnostic and contrast agents, used on or administered to person to diagnose, treat, or prevent disease or other abnormal conditions…" [29].

JCAHO's National Patient Safety Goal #8 Medical Reconciliation Act requires documentation of any patient use of herbal remedies, vitamins, nutriceuticals, over-the-counter drugs, just like any other medication [30]. This is a standard that applies to all accredited hospitals in the United States.

Details can be found at http://www.jointcommission.org/standards_information/npsgs.aspx. The patient or a family member/caregiver should be asked to provide a list of the types and amounts of dietary supplements that the patient uses or has used in the recent past, and this information should be entered into the chart and electronic medical records as part of the dietary assessment. It is important for all health professionals who see the patient to query him or her about dietary supplement use; some patients are reluctant to tell the doctor but are willing to share their usage patterns with dietitians. Everyone is responsible to collect this information, and so dietitians, nurses, and pharmacists as well as physicians should be alert to these issues. When electronic medical records are developed, a question on use of dietary supplements should be included, and the supplements that are used should be named in the medical record.

2 Outpatient Settings

Box 2.1 provides some guidelines for health professionals when they are considering making recommendations to patients on dietary supplements. During nutritional assessment, all patients should be asked about their use of dietary supplements: what supplements they use, how much they use, how often, and where they obtained them (e.g., drugstore, friends, the Internet). Replies should be written in the medical record. Dietary supplement use should be included in diet history and in the calculation of nutrient intake. Some food frequency questionnaires and food checklists include items on the intake of the most commonly consumed dietary supplements, but these may not include less commonly used products or supplements used only occasionally that may also be important to health. Therefore, it is prudent to probe for additional supplement use and to encourage patients to write these dietary supplements on their questionnaires. If questions remain or there is need for further documentation, the patient can be asked to keep a supplement intake record that he or she can bring to the next visit. One useful way to elicit further information about dietary supplement use from ambulatory patients who report very high use but cannot remember details of what they take at home is the "brown bag" technique. The patient is asked to bring in a bag all of the dietary supplements and medications that he or she uses to the next visit. The doses and types of dietary supplements and other medications can be recorded in the chart, and their potential impact can be taken into account in dietary assessment and assessment of possible supplement−nutrient interactions. NIH's brochure "Dietary Supplements: What You Need to Know" provides answers to some questions about dietary supplement use and medications; it can be downloaded and printed from the ODS website at https://ods.od.nih.gov/HealthInformation/DS_WhatYouNeedToKnow.aspx.

TABLE 2.2 Dietary Supplement Assessment Methods

Method	Advantages	Disadvantages	Comments
Pill inventories	Actual labels available and can be examined, doses recorded.	Patients/clients may forget or refuse to bring supplements when requested, or only produce socially acceptable/legal products. May change dietary supplement use reporting. UPC codes for dietary supplements are not unique and the formulations in a given specific UPC-coded product may change.	Technique is commonly used in studies of medication use. A variant is to ask the patient/respondent to provide drug or grocery receipts, and if these include UPC codes it may be possible to identify the supplement.
Diet records	May be useful in clinical settings with willing patients to improve adherence and obtain specific details about usage patterns. Provides actual record of intake going forward. Respondent has the bottle from which he or she records information and recall of items used is not necessary. May provide useful contextual information for improving adherence.	May change eating or dietary supplement use behavior, especially if supplements have been prescribed. The process is extremely time-consuming for the recorder, usual intake may only be revealed with use over many days and forgetting to record is common.	Usually, the record includes food and drink as well as dietary supplement use; can be expanded to also cover other medications when drug–supplement or food–supplement interactions are of interest.
Frequency questionnaires	Retrospective so does not affect food consumption. Lists may help to prod memory and make recall easier. May provide an estimate of usual intake. Quick to fill out. The standardized format makes it useful for large-scale studies. Important that write-ins can be accommodated.	Lists not usually complete and may be very nonspecific. For some condition-specific and other supplements, use is very infrequent and may not show up if the window of recall is approximately 30 days. Semiquantitative dietary supplement intake forms are not quantitatively precise.	Frequency questionnaires for dietary supplement use range from simple checklists for categories of dietary supplements (e.g., MVM supplements, single vitamin or mineral supplements, and others) or specific supplements to semiquantitative questionnaires that tap not only frequency but also amount used.
24-Hour recalls	Retrospective so does not affect food consumption. Quantifies intake, usually easy for patient to recall. May point to problems with timing or other aspects of supplement use. Some computerized dietary assessment programs include a dietary supplement assessment module so that both food and supplement intakes can be ascertained.	Relies on memory and some items may be forgotten or individual may not be able to provide sufficient detail about the exact supplement name, dose, etc. Many days are needed to estimate usual intake. May be useful clinically but more difficult to use in large studies in which standardization is necessary.	Individual is usually asked to provide his or her intake of dietary supplements as well as food and drink that has been consumed in the past 24 hours.
Diet histories	Food intake is not changed because method is retrospective. However, respondent may not recall usual pattern of supplement intake or may not have a usual pattern. Permits obtaining information on total diet.	Recall is involved and memory may be faulty. The amounts are usually not precise. Time-consuming for both investigator and respondent.	Individual provides the professional with information on "usual" diet.
Brief dietary supplement assessment forms	Do not change eating behavior because they are retrospective. Focus solely on dietary supplement use. Easy to fill out and inexpensive.	Only a small number of supplements or foods or both can be asked about. Often, information on dose, type of supplement, timing, etc. is not provided.	Individual is asked to respond with "usual" dietary supplement intake.

(*Continued*)

TABLE 2.2 (Continued)

Method	Advantages	Disadvantages	Comments
Blood and urine	Does not change supplement use because it is retrospective. If the only source of the biomarker is the dietary supplement, it is possible to state with certainty that the product was consumed.	Not all micronutrients, other nutrients, or botanicals have easily identifiable biomarkers. Method is not quantifiably precise.	A blood (e.g., folic acid) or urine (e.g., creatine) biomarker is used to ascertain intake, either in conjunction with or instead of usage data on dietary supplements.

BOX 2.1 Guidelines for Health Professionals When Considering Recommendations for Dietary Supplements

- Is there a shortfall in nutrient intakes from recommendations when all sources of nutrients (food, beverages, and medications such as antacids that contain nutrients and dietary supplements) are taken into account? If so, try to satisfy needs first by the use of usual foods or fortified foods, or are dietary supplements in order?
- Does the supplement contain more than 100% DV and especially more than the upper level of nutrient intake recommended? If so, choose a supplement that contains lesser amounts; there is no known benefit to consuming more than the Recommended Dietary Allowance (or Adequate Intake) of a particular nutrient.
- Is the dietary supplement standardized and certified by an accredited source, such as the U.S. Phamacopoeia (USP) National Formulary (NF) showing that the USP standards for identity, purity, packaging, and labeling were followed? If so, they are labeled with the USP/verified symbol. The USP website is http://www.usp.org.
- Is the product fresh? If so, it has not exceeded its expiration date on the label.
- Does the supplement contain labeled amounts of nutrients or other constituents? Currently, there is no single source that provides verifications for label claims for nutrients and other constituents in supplements. Some useful information may be available if the product has been tested by ConsumerLab.com, USP, or NSF, and found to contain amounts of constituents claimed on the label. An Informed Choice label is certified for sports specific supplements. It can be accessed at http://informed-choice.org/.
- Is the dietary supplement reasonable in cost and within the patient's economic means?
- Has the patient read the patient package insert and cautionary directions and dosage limits on the label? If so, the patient should be alert to adverse reactions specified and report them to his or her physician if they arise.
- Does the dietary supplement claim to prevent or cure a condition or disease? By law, dietary supplements cannot be claimed to prevent or cure disease, and so the claim is not supportable and conventional methods for doing so should be sought.
- Is the dietary supplement safe? Certain dietary supplements such as ephedra (Ma huang) have been declared unsafe by the FDA and the U.S. Department of Health and Human Services. Check the CFSAN website at FDA for updates on other products.
- Is the dietary supplement efficacious? If so, authoritative bodies such as the Food and Nutrition Board, of the Division of Medicine, National Academy of Medicine, the Consensus Conferences of the Agency for Healthcare Research on Quality should have indicated that such uses are safe and effective. The website www.ods.od.nih.gov summarizes information on many studies of safety and efficacy.

Source: *Adapted and modified from R.B. Costello, M. Leser, P.M. Coates, Dietary supplements for health maintenance and risk factor reduction, in: C.W. Bales, C.S. Ritchie (Eds.), Handbook of Clinical Nutrition and Aging, Humana Press, Totowa, NJ, 2005. [59].*

For patients with "smart" devices such as phones and tablets, there are numerous applications ("apps") available for keeping track of dietary supplements and medications. Each app provides different features, including tracking, reminders, background information, and the ability to share the information with a health professional at a later time.

Sources such as the "Healthcare Professional's Guide to Popular Dietary Supplements" [31] and "A Healthcare Professional's Guide to Evaluating Dietary Supplements" [32] may be helpful for obtaining estimates of ingredients and content.

For those in healthcare facilities, clinical pharmacists are an underutilized resource for questions that may arise on supplements, particularly if there is reason to suspect that a supplement may generate adverse reactions.

C Estimating Dietary Supplement Intake

Once the patient's reported intake has been obtained, the information must be evaluated. When total nutrient intake is needed, nutrients from food, beverages, and nutrient-containing supplements must be added together to get an estimate of the individual's total dietary intake. For some, this may include capturing intakes not only from pills and tablets but from food bars or sports drinks that are often very highly fortified and sold as supplements.

In large populations, especially if adequacy or excess are of interest, it is best to present intakes of supplement users separate from those of nonusers. Means for the entire population are less informative of such problems.

D Assessment of Dietary Supplement Intake in Some Large-Scale National Surveys

1 National Health and Nutrition Examination Survey

NHANES is a population-based survey to assess the dietary intakes, health, and nutritional status of noninstitutionalized adults and children in the United States. Approximately, 5000 people are surveyed each year in five communities nationwide. In addition to food intake, information on dietary supplement use (frequency, amount, and duration) is collected from respondents during the interview in their households. The dietary supplements include vitamins, minerals, other prescription and nonprescription dietary supplements, and antacids (a major source of calcium) that have been taken in the past month. Prescription dietary supplements are also listed but in separate files. During the household interview, details on supplement use are collected. Respondents who say that they have taken dietary supplements are then asked to provide the supplement containers. Approximately two-thirds of them do so. Supplement containers are viewed, and the interviewer records from the product label the name, strength of the ingredient (for certain vitamins and minerals), and other information. During the household interview, details on supplement use, such as how long the product has been used, how often it was taken during the past month, and how much was taken, are established [33]. Since 2007, NHANES has also collected two 24-hour recall interviews as well as interval estimates during the past 30 days. An advantage of NHANES is that estimates of usual intakes of the nutrients in foods as well as in dietary supplements can be obtained. These are necessary to estimate the proportion of the population under the estimated average requirement or over the upper tolerable level (UL). Since NHANES collects both information on dietary intake and supplement use, along with anthropometric, biochemical, clinical reports of the presence of various conditions, it is possible to obtain information on the associations between supplement use, total intakes of the ingredient in question, and health status. Since the data are cross-sectional, causal inference is weak, but hypotheses generated from it can be tested in other populations to obtain more definitive answers. General questions on the motivations for supplement use are included in NHANES.

2 National Health Interview Survey

The National Health Interview Survey (NHIS) is a very large population-based survey that has periodically obtained information on dietary supplement intake in years when complementary and alternative medicine (CAM) supplements were included (2002, 2007, and 2012 survey years, with another supplement likely in 2018) [34]. The NHIS now has combined data on 88,962 adults aged 18 and over. The large sample size allows for estimation of the use of dietary supplements, including seldom consumed supplements, by a wide variety of population subgroups. The data products are available at the NHIS website: http://www.cdc.gov/nchs/nhis/index.htm. These data are useful in assessing the use of supplements in the context of CAM use [10,34]. Unfortunately, no information on dietary intake, biochemical, anthropometric or clinical data is collected simultaneously. Nevertheless, the survey has been used in studying issues such as use of dietary supplements in adults who reported that they suffered from gastrointestinal conditions [35]. The advantage is that the survey obtains detailed information on motivations, associations with conventional medical treatments and costs, as well as some information on dietary supplement use. Although the sampling frame is complicated, the questions asked about CAM and use of dietary supplements provide material not available in other national surveys. The disadvantage is that supplement dose information, food intake, and health indices are not included and so little can be concluded about health status other than that provided by respondent report. The NHIS will be updated in 2018 to better meet the needs of data users. Questions will focus on the health of one sample adult and one sample child per household, and information on other members of the household will be limited. A fixed core questionnaire will be used annually, and additional core areas will rotate on or off the questionnaire. Topics and items are currently being considered for the sample adult and child interviews; it is unclear if information on dietary supplement use will be collected.

3 National Cancer Institute's Diet and Health Questionnaire: A Public-Use Semiquantitative Food Frequency Questionnaire

The National Cancer Institute (NCI) has developed a semiquantitative food frequency questionnaire that is publicly available. The questionnaire can be reprogrammed to add specific dietary supplements. As issued, it includes specific questions on multivitamins, herbals, antioxidant supplements, and vitamins A, C, and E use during the past year and also a question on how long calcium-containing supplements or antacids have been used. There are also limited queries on approximately 10 vitamins and minerals, as well as fatty acids, and approximately 24 different herbals and botanicals that are frequently used. The questionnaire includes both foods and supplements, and it can be used for many purposes, not simply cancers. The 2006 version of the Diet and Health Questionnaire (DHQ) was validated using a checklist approach and was found to be an improvement over previous versions of the NCI questionnaire known as the 1992 NCI/Block questionnaire [36]. There have been no such validation studies with the DHQ II. However, validation findings are unlikely to be greatly modified by the minimal modifications to the food list and the updated nutrient database. The DHQ can be accessed at http://riskfactor.cancer.gov/dhq2.

4 Other Instruments for Assessing Dietary Supplement Intake in Epidemiological Studies

Many different proprietary semiquantitative questionnaires that are variations of food frequency questionnaires with additional questions on dietary supplements exist for assessing dietary supplement intakes in epidemiological studies of various large cohorts. However, the questions vary from one questionnaire to another, depending on the focus of the study. The core components of all the questionnaires are the U.S. Department of Agriculture's (USDA's) Food Composition tables, combined into groups to correspond to items or recipes on the questionnaires. The lists of dietary supplements on the various semiquantitative food frequency questionnaires vary greatly from one another. In the future, it would be helpful to develop common questions or lists that would improve comparability from one study to the next. In 24-hour recall programs or data analysis systems, often questions to probe dietary supplement use are not included. Data on dietary supplement composition from self-entered values, when available, are usually obtained from manufacturers; they can now also be obtained from the NIH DSLD available on the web. Total intakes reported should reflect intakes not only from food but also from the specific dietary supplements that were queried.

There are many examples of tools for adults that query dietary supplements as well as foods; those for children and infants are fewer in number. The questionnaires require specific food composition and dietary supplement databases to be analyzed, and these are not generally in the public domain. Adult questionnaires that query some dietary supplements include the Harvard (Willett) semiquantitative food frequency questionnaire, different versions of which have been used in a number of studies; the various semiquantitative food frequency questionnaires developed by Block's group now available at Nutriquest.com; the University of Hawaii Cancer Center's multiethnic cohort questionnaire (a particularly detailed and well-validated questionnaire); the Women's Health Initiative semiquantitative food frequency questionnaire; the Women's Health Study questionnaire; the National Institute of Environmental Health Sciences' Sisters Study Questionnaire; and the American Cancer Society's food frequency questionnaire. The availability of these questionnaires varies, and use depends on obtaining permission from the owners of the questionnaires and their willingness to collaborate with other investigators. Some university research groups, such as the dietary assessment groups at the University of Washington−Fred Hutchinson Cancer Center, Harvard University, and the University of Hawaii Cancer Center, will permit their questionnaires to be used, and some will even analyze study results for a fee.

The Fred Hutchinson Cancer Research Center at the University of Washington was the Data Coordinating Center for the Women's Health Initiative. It has developed a module based on that work and refined it, for two other large NIH-sponsored clinical trials of adults, the SELECT (Selenium and Vitamin E Cancer Prevention Trial) and the VITAL (Vitamin and Lifestyle Cohort Study). It has made them available to outside groups and will process them for a fee. The VITAL study questionnaire has been further revised. The questionnaires have lists of both nutrient supplements and herbal/botanical supplements, and they come in both male and female versions. The center's website (http://sharedresources.fredhutch.org/core-facilities/nutrition-assessment) describes the services it provides to outside users.

Although use of dietary supplements is widespread, intakes from supplements, particularly by subgroups in the populations who may be heavy users, are difficult to quantify. The Supplement Reporting (SURE) study at the University of Hawaii Cancer Center developed a unique inventory method to quantify dietary supplement use. Interviewers visited participants' homes to record supplement purchase and the number of pills in each supplement bottle every 3 months over a year. The resulting inventory method markedly improved the in-depth measurement of supplement use [37]. Based on the results of the SURE study, a 1-page questionnaire for dietary supplement use (SURE-QX; Fig. 2.1) has been developed and is available

DID YOU TAKE ANY DIETARY SUPPLEMENTS DURING THE PAST YEAR, AT LEAST ONCE A WEEK?

① YES ↓ If yes, did you take any of the following? ② No

VITAMIN TYPE	HOW OFTEN?			FOR HOW MANY YEARS?			
	1 to 3 times a week	4 to 6 times a week	Once a day	1 year or Less	2 to 4 years	5 to 9 years	10 years or more
MULTIPLE VITAMINS Regular one-day-type, Centrum® or Thera-type	①	②	③	①	②	③	④
B-complex or Stress-tab type	①	②	③	①	②	③	④
SINGLE SUPPLEMENTS Vitamin C	①	②	③	①	②	③	④
Vitamin E	①	②	③	①	②	③	④
Folic acid, folate	①	②	③	①	②	③	④
Vitamin B-12	①	②	③	①	②	③	④
Vitamin B-6	①	②	③	①	②	③	④
Calcium, alone or combined with something else such as in a bone health supplement OR in an antacid	①	②	③	①	②	③	④
Vitamin D, alone	①	②	③	①	②	③	④
Selenium	①	②	③	①	②	③	④
Iron	①	②	③	①	②	③	④
Zinc	①	②	③	①	②	③	④
Fish oil or omega-3 fatty acids	①	②	③	①	②	③	④
Flaxseed	①	②	③	①	②	③	④
Garlic, as a pill, tablet, or capsule	①	②	③	①	②	③	④
Glucosamine, alone or combined with something else	①	②	③	①	②	③	④
Coenzyme Q-10	①	②	③	①	②	③	④
Saw Palmetto	①	②	③	①	②	③	④

IF YOU TOOK VITAMIN C OR VITAMIN E:

When you took VITAMIN C, how much did you usually take?
- F 250mg or less
- F 300 to 500mg
- F 600 to 1000mg
- F More than 1000mg

When you took VITAMIN E, how much did you usually take?
- F 200IU or less
- F 250 to 400IU
- F 450 to 1000IU
- F More than 1000IU

ID#._____

FIGURE 2.1 Sure-QX: short supplement questionnaire developed by the University of Hawaii Cancer Center, Honolulu, Hawaii. *From The Supplement Reporting (SURE) Study, University of Hawaii, Cancer Research Center of Hawaii, J. Food Compost. Anal. 22 (Suppl. 1) (2009) S83–S87.*

for use by researchers. The longer University of Hawaii Cancer Center food frequency questionnaire has 180 foods and 10 supplements; it is available to investigators, who can purchase it from the center and then return it for processing into nutrient intakes. The analysis provides separate variables for the intake of nutrients from food ($n = 54$) and the intake of nutrients from supplements ($n = 22$). A less extensive list of supplements is provided in the SURE-QX questionnaire described previously. The SURE-QX questionnaire has detailed questions and many defaults to permit more precise and accurate information on the dietary supplements that are used. This questionnaire also includes many supplements used by Asian-Americans.

Other semiquantitative questionnaires are available from commercial services. Perhaps the best known is the Nutritionquest group, which provides the Nutritionquest or Block semiquantitative food frequency questionnaire; it can be purchased and accessed at http://www.nutritionquest.com. The basic 2005 food frequency questionnaire has approximately 110 foods and also items on multiple vitamin supplements, single vitamins and minerals, and

one item on herbals. The latest version of this instrument was developed in 2014. Upon request and for a fee, the Block questionnaires can be tailored for individual research purposes. The Block Nutritionquest group has also developed a brief calcium/vitamin D screener that includes 19 foods and 3 supplements as well as questions to adjust for food fortification practices. Another Block screener is the Block Folic Acid/Dietary Folate Equivalents Screener, which is based on NHANES 1999−2001 dietary recall data. It includes 21 questions and provides separate estimates of total, supplement, and food-only intakes [38]. A questionnaire is available for dialysis patients consisting not only of foods but also supplements and liquids. Recently a sodium screener has been developed; fat/sugar/fruit/vegetable screeners are also available. The Block Soy Foods Screener focuses on 10 food and supplement items and was designed to measure intakes of daidzein, genistein, coumestrol, and total isoflavones. The drawback is that the lists of dietary supplements are usually short.

E Other Instruments Used for Assessing Dietary Supplement Intake in Clinical Research Studies

Many other techniques have been used to obtain information on dietary supplement intakes. Serial random 24-hour recalls that included foods as well as supplement intakes were used successfully in the Women's Intervention Nutrition Study, a large randomized trial of diet as adjuvant therapy in women who had been treated for breast cancer [39]. In the Hemodialysis study of patients undergoing renal replacement therapy, food and dietary supplement intake were assessed using a 2-day diet diary-assisted recall technique, in addition to medication inventories and other techniques to check on adherence to use of high-dose B vitamin supplements [40].

III DIETARY SUPPLEMENT COMPOSITION DATABASES FOR ANALYSIS OF DIETARY SUPPLEMENT INTAKE

The analysis of dietary supplement intakes ideally requires complete analytically verified tables of dietary supplement composition by chemical analyses, which still do not exist for all nutrients and even more rarely for other bioactives in supplements. Therefore, results tend to be imprecise and inaccurate, particularly for intakes of some of the botanical ingredients in dietary supplements. The situation is slowly changing, but dietary supplement databases are still incomplete with respect to both how representative they are of the universe of dietary supplements marketed and sold in the United States and how well the levels of ingredients are documented. Currently, virtually all of them rely on label claims rather than analytically verified data. For dietary supplements and many highly fortified processed foods that lack analytical data on micronutrients, intake estimates obtained from product label declarations are likely to be biased. Overages are likely, especially for vitamins because labeling regulations require that the actual nutrient content of products be equal to or greater than the declared level on the label, after taking into account processing effects and shelf life losses [41].

A Dietary Supplement Label Databases

1 NHANES Survey Label Database

The composition of dietary supplements consumed in the NHANES is available at the National Center for Health Statistics website, although the primary purpose of the database is to store information on nutrients taken from the dietary supplement labels collected from NHANES respondents. NHANES research nutritionists obtain additional label data for the dietary supplement database by contacting manufacturers and distributors, company websites, and other Internet sources. Changes in supplement composition are tracked and entered into the database when reformulations are identified. The NHANES label database is publicly available and permits nutrition scientists to better assess total intakes of nutrients from all sources than ever before. However, it has its limitations. Only supplements that were used by respondents in the survey are provided in the database. Approximately 10,000 respondents are included in each NHANES data release. Although this may seem like a large number of respondents, for rarely used supplements, there may be few or no users who respond. Only levels of nutrients are noted, although the names of other ingredients are recorded as well. The quantitative data on nutrients that it provides rely on nutrient content declarations on the labels and are not analytically verified. Because it is a violation of the law to declare levels of a nutrient on the label as being more than what is provided, manufacturers tend to add more than the declared label value to many products. The amount added depends on the particular nutrient in question, its stability, cost, bulk, and other characteristics; there is no single "correction factor" that can be used. The supplements that are reported in NHANES during the most recent interview cycle are released every 2 years. Unfortunately, supplements on the market change rapidly, and many of the products may not be on the market at the time the database is accessed. Default values are also included in the database because many respondents are unable to supply the exact supplement or strength that was consumed, although some information is available. Because NHANES uses a nationally

representative sampling procedure, defaults developed with the NHANES data may be useful in other surveys as well, particularly if it is not possible to collect data with this level of detail. The defaults are based on the frequency of supplements reported in the latest 2-year NHANES release that is available, as well as on manufacturer information on sales. For example, default matches for adults include matching multivitamins to multivitamin minerals, as well as single ingredient formulations such as vitamin A to 8000 IU, vitamin C to 500 mg, vitamin B_6 to 100 mg, vitamin D to 400 IU, vitamin E to 400 IU, folic acid to 400 μg, calcium to 500 mg, iron to 65 mg, and zinc to 50 mg.

2 Dietary Supplement Label Database

The DSLD from the NIH contains information taken from the labels of approximately 60,000 dietary supplement products available in the U.S. marketplace. Launched in 2013, this free resource will grow to include most of the different dietary supplement products sold. The DSLD is available at http://www.dsld.nlm.nih.gov/dsld.

The DSLD offers these features:

- *Quick Search*: Search for any ingredient or specific text on a label.
- *Search for Dietary Ingredients*: An alphabetical list of ingredients is provided.
- *Search for Specific Products*: An alphabetical list of products is provided.
- *Browse Contact Information*: Search by supplement manufacturer or distributor.
- *Advanced Search*: Search by using a combination of search options including dietary ingredient, product/brand name, health-related claims, and label statements.

The DSLD provides product information that can be organized and searched by users. Research scientists, for example, will use the DSLD to determine total nutrient intakes from food and supplements in populations they study. Healthcare providers can learn the content of products their patients are taking. The DSLD is a collaborative project of the ODS and the NLM at NIH, with input from many federal stakeholders including most NIH institutes and centers, the USDA's Agricultural Research Service, the CDC's National Center for Health Statistics Division of Health and Nutrition Examination Surveys, and the U.S. FDA's Center for Food Safety and Applied Nutrition.

3 Natural Medicines Comprehensive Database

This database, formerly called the Natural Medicines Database, provides objective and reliable information for clinicians based on an evidence-based, consensus-based, peer-reviewed procedure with reproducible grading scales. Access is by subscription, and it is available at Natural Medicines Database, https://naturalmedicines.therapeuticresearch.com. Subscribers also have access to a compendium of evidence-based reviews on herbs and dietary supplements that is available online as well as in print. Information also includes risk assessments. It is used in the Veterans Administration, the Department of Defense (DOD), and other agencies within government. This database provides not only an in-depth information about the composition of dietary supplement products and ingredients but also ratings for the safety and effectiveness of products along with the uses, benefits, side effects, drug interactions, etc. of the ingredients found in dietary supplement products.

4 Trade Associations: Council for Responsible Nutrition and the Natural Products Association Database

The Council for Responsible Nutrition (CRN), a trade association for the dietary supplement and functional food industry, announced in July 2016 that it had retained UL, a global independent safety science company, to develop and administer the dietary supplement product registry that CRN hopes "will help create a fuller picture about the dietary supplement industry for industry regulators and serve retailers as a one-stop shop to help compare product labels." The online product registry will include full label information for dietary supplements and be accessible via the web. CRN member companies will be required as a condition of membership to input all their product labels into the product registry by July 2017, and all dietary supplement companies will be strongly encouraged to do the same.

The Natural Products Association (NPA) is a trade association (formerly the National Nutritional Foods Association) and an associated foundation. The NPA operates a two-part quality assurance program that includes a third-party certification program for good manufacturing practice (GMP) standards as determined by NPA based on a dialogue between suppliers and others. Those who meet the standard and pass audits can use the NPA logo. Members can also participate in the TruLabel program, which includes data on ingredients in members' product labels. The database is not available to the public. A description is available at http://www.npainfo.org/NPA/EducationCertification/TruLabelProgram.aspx.

5 Other Label Databases

Several other private compilations of dietary supplement label information are becoming available and may be purchased in the future.

B Dietary Supplement Databases With Verified Chemical Analyses

Although databases for dietary supplements are based on chemical analyses, usually the data on the label are proprietary and analytic data are disclosed only at the manufacturer's discretion. Several publicly available chemically analyzed dietary supplement databases now exist, but they contain only a few types of products, are not always based on representative numbers of products, and some are proprietary. The major ones are described here.

1 USDA Dietary Supplement Ingredient Database

The ODS at NIH collaborated with the USDA to develop an analytically substantiated dietary supplement ingredient database (DSID) for the micronutrients and eventually for other constituents as well in popular highly consumed supplements. Initial efforts focused on MVM supplements because these are the most commonly consumed supplements by Americans [41]. The database is now publicly available for adult MVM, child MVM, prenatal over-the-counter MVM, and omega-3 fatty acids. In the future, calcium-vitamin D supplements and green tea supplements will be assessed. The DSID is based on chemical analysis of the nutrient content in dietary supplements, compared with label-reported ingredient levels. For example, the DSID provides estimated levels of 18 vitamin and mineral ingredients derived from analytical data for 115 representative unspecified adult MVM supplements. The DSID also includes a calculator, which allows the consumer to compare his/her product's contents to that of a representative sample of similar products in the United States. It is available on the web free of cost at http://www.dietarysupplementdatabase.usda.nih.gov/index.html.

2 Consumerlabs.com

ConsumerLab.com, LLC, is a provider of independent test results and information to help consumers and healthcare professionals evaluate dietary supplements and other health, wellness, and nutrition products. Data are published only on products that have been tested. The products are bought off the shelf in consumer outlets and chemically analyzed for various substances of interest. ConsumerLabs does not publish a comprehensive database that is publicly available. However, a subscription to its reports is available for a reasonable cost at http://www.consumerlab.com.

3 NSF

NSF is an independent, not-for-profit testing organization offering product testing of dietary supplements in its NSF/American National Standards Institute. The organization does not simply evaluate test data submitted by manufacturers or analyze a single sample of a product and approve it; rather, NSF conducts its own product testing in its accredited laboratories. The three main components of the NSF Dietary Supplements Certification Program are verification that the contents of the supplement actually match what is printed on the label, assurance that there are no ingredients present in the supplement that are not openly disclosed on the label, and assurance that there are no unacceptable levels of contaminants present in the supplement. The major disadvantage of the values published by NSF is that they do not constitute a comprehensive database. Only products that have been certified are included in the database. Currently, approximately 590 products from 79 companies are certified on the NSF website at http://info.nsf.org/Certified/Dietary/.

C Computerized Dietary Assessment Programs that Include Dietary Supplements

1 University of Minnesota Dietary Supplement Module

Some computerized dietary assessment programs include dietary supplements in the interview and also have databases on their composition. For example, the University of Minnesota's Nutrient Data System has developed a dietary supplement assessment module that can be used in conjunction with existing software so that nutrient intake from both foods and dietary supplements may be quantified. This can be accessed at http://www.ncc.umn.edu/products/.

2 NCI's Automated Self-Administered 24-Hour Dietary Recall

The Automated Self-Administered 24-Hour Dietary Recall (ASA24) is a free web-based software tool, developed by the NCI and Westat, that enables automated self-administered 24-hour recalls and food records. The system includes an optional dietary supplement module that allows respondents to report supplements based on the NHANES dietary supplement data set. The ASA24 Respondent website guides the participant through the completion of either a 24-hour recall for the previous day (from midnight-to-midnight or for the past 24 hours) or for a single or multiple day food record and a Researcher application for researchers to manage study logistics and obtain data analyses. The system allows for probing (based on USDA's automated multiple pass method), coding, and the calculation of dietary intakes using the USDA's Food and Nutrient Database for Dietary Studies and the NHANES Dietary Supplement Database. The tool

is a highly interactive web-based application employing approximately 1500 unique probe question pathways and more than 12,000 food images to estimate portion size. ASA24 can be used by researchers for epidemiologic, interventional, behavioral, or clinical research. Clinicians may also find it useful for dietary assessment or nutrition counseling, and educators may find it to be a useful teaching tool. Detailed information about ASA24, including information on registering a study and a demonstration of the Respondent application, is available at http://epi.grants.cancer.gov/asa24/. A listserv is available on ASA24 at http://epi.grants.cancer.gov/asa24/. The content from the current website is being moved and so links and bookmarks may be changed in the future.

3 Other Computerized Dietary Assessment Programs

Other computerized dietary assessment programs permit the addition of supplement information to the database even if they are not included in the food composition database, but none yet provide complete lists of the most commonly used supplements in the software package.

IV THE DIETARY SUPPLEMENT LABEL

Many of the labels for dietary supplements are now included in the NIH's DSLD described earlier in this chapter and can be downloaded if that is desired. FDA regulations require certain label information on dietary supplements, including a descriptive name of the product stating that it is a "supplement"; the name and place of business of the manufacturer, packer, or distributor; a complete list of ingredients; and the net contents of the product. In 2016, FDA announced the final rule for an update of the food label, which will include updated Daily Values (DVs) and other changes. Since, under U.S. law, dietary supplements are considered to be foods, the Supplement Facts label will change accordingly. Fig. 2.2 shows changes to the Nutrition Facts Label on food and the new Facts label as of 2016.

The regulations are described in-depth in the FDA's Dietary Supplement Labeling Guide (accessible at http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/dietarysupplements/ucm2006823.htm). FDA has issued regulations for GMP that touch upon such topics as verification of identity, purity, strength, and supplement composition, and these are soon to be in effect.

Nutrition Facts

Serving Size 2/3 cup (55g)
Servings Per Container About 8

Amount Per Serving

Calories 230	Calories from Fat 72
	% Daily Value*
Total Fat 8g	**12%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 160mg	**7%**
Total Carbohydrate 37g	**12%**
Dietary Fiber 4g	**16%**
Sugars 1g	
Protein 3g	
Vitamin A	10%
Vitamin C	8%
Calcium	20%
Iron	45%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily value may be higher or lower depending on your calorie needs.

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Nutrition Facts

8 servings per container
Serving size **2/3 cup (55g)**

Amount per serving
Calories **230**

	% Daily Value*
Total Fat 8g	**10%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 160mg	**7%**
Total Carbohydrate 37g	**13%**
Dietary Fiber 4g	**14%**
Total Sugars 12g	
Includes 10g Added Sugars	**20%**
Protein 3g	
Vitamin D 2mcg	10%
Calcium 260mg	20%
Iron 8mg	45%
Potassium 235mg	6%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

FIGURE 2.2 Former and new (2016) supplement facts label. *Taken from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm385663.htm#images.*

A Dietary Supplement Label: Ingredients

1 Differences Between Food and Dietary Supplement Labels

The Supplement Facts panel on dietary supplements must list dietary ingredients that have, as well as those that do not have, recommended daily intakes (RDIs) or daily reference values (DRVs or DVs). Listing the source of a dietary ingredient on the label is optional. In contrast, sources of a dietary ingredient and ingredients without RDIs or DVs are not permitted on the food Nutrition Facts label. Also, the part of the plant from which a dietary ingredient is derived must be listed on the Supplement Facts panel for dietary supplements, although it cannot be listed on the food label. In contrast, the Supplement Facts panel does not permit listing of "zero" amounts of nutrients, although the Nutrition Facts panel for food requires it. The percent DV (i.e., % DV or the Reference Daily Intake or DRV) of a dietary ingredient contained in a serving of the product must be declared for all ingredients for which there are DVs except protein. Supplements for infants, children younger than 4 years, and pregnant and lactating women do not require this, however.

2 Supplement Facts Label

The panel must list the names and amounts of the dietary ingredients present in the product, the serving size, and servings per container. A serving for a dietary supplement is the maximum amount recommended for consumption at one time or, if recommendations are not given, 1 unit (e.g., tablet, capsule, packet, or teaspoon). Thus, if the label says to take one to three tablets with breakfast, the serving size is three tablets.

3 Ingredient List

Other dietary ingredients that do not have DVs are also listed in the Supplement Facts panel after the ingredients that do have them, and in addition with their correct botanical (Latin) names. They are also listed by their common or usual names and must be accompanied by their weight per serving.

B Dietary Supplement Label: Claims

Box 2.2 describes the three categories of claims that can be used on dietary supplements: health claims, nutrient content claims, and structure–function claims.

V AUTHORITATIVE INFORMATION AND RESOURCES ABOUT DIETARY SUPPLEMENTS

A Office of Dietary Supplements, National Institutes of Health

The ODS at NIH has as its mission to strengthen knowledge and understanding of dietary supplements by evaluating scientific information, stimulating and supporting research, disseminating research results, and educating the public about the efficacy and safety of dietary supplements in order to foster enhanced quality of life and health for the U.S. population. Their website contains much useful information for health professionals and can be accessed at http://ods.od.nih.gov.

1 NIH RePORTER

NIH provides statistics and data about its applications and grants through the Research Portfolio Online Reporting Tools (RePORT) website. The NIH RePORTER database is available to all public users at http://exporter.nih.gov/. Researchers can use a keyword search function to search the database for grants supporting research on specific nutrients or dietary supplements by current year or by a specified year as well as search for a specific investigator's work.

Clinicaltrials.gov, maintained by NIH, is a list of all registered clinical trials, including those using dietary supplements. The purpose of the study, dosing levels, and other details are provided. This resource can be accessed at https://clinicaltrials.gov/.

2 Other ODS Resources

ODS also provides a number of other authoritative health information materials on its website. Of particular use to health professionals are dietary supplement fact sheets that also include both consumer English and Spanish versions (http://ods.od.nih.gov).

B Food and Drug Administration

1 Center for Food Science and Nutrition (for Health Claims)

The FDA's Center for Food Science and Nutrition's (CFSAN's) website and its ODS have a variety of materials on dietary supplements, including recent recalls, frequently asked questions, and some materials for consumers. These resources can be accessed at www.fda.gov/food/default.htm.

BOX 2.2 Claims That Can Be Used on Dietary Supplements

Health Claims

Health claims describe a relationship between a food, food component, or dietary supplement ingredient and reducing risk of a disease or health-related condition. A "health claim" definition has two essential components: (1) a substance (whether a food, food component, or dietary ingredient) and (2) a disease or health-related condition. A statement lacking either one of these components does not meet the regulatory definition of a health claim.

FDA has oversight in determining which health claims may be used on a dietary supplement label. Its authority comes from several laws:

- *NLEA Authorized Health Claims*: The Nutrition Labeling and Education Act (NLEA) of 1990, the Dietary Supplement Act of 1992, and the Dietary Supplement Health and Education Act of 1994 (DSHEA) provide for health claims used on labels that characterize a relationship between a food, a food component, dietary ingredient, or dietary supplement, and risk of a disease provided the claims meet certain criteria and are authorized by an FDA regulation. FDA authorizes these types of health claims based on an extensive review of the scientific literature, generally as a result of the submission of a health claim petition, using the significant scientific agreement standard to determine that the nutrient–disease relationship is well established. For an explanation of the significant scientific agreement standard, see the FDA website.
- *Qualified Health Claims*: FDA permits the use of qualified health claims when there is emerging evidence for a relationship between a food, food component, or dietary supplement and reduced risk of a disease or health-related condition. When this is the case, the evidence is not well enough established to meet the significant scientific agreement standard required for FDA to issue an authorizing regulation. Qualifying language is included as part of the claim to indicate that the evidence supporting the claim is limited. Both conventional foods and dietary supplements may use qualified health claims. FDA uses its enforcement discretion for qualified health claims after evaluating and ranking the quality and strength of the totality of the scientific evidence. Although FDA's "enforcement discretion" letters are issued to the petitioner requesting the qualified health claim, the qualified claims are available for use on any food or dietary supplement product meeting the enforcement discretion conditions specified in the letter. FDA has prepared a guide on interim procedures for qualified health claims and on the ranking of the strength of evidence supporting a qualified claim. Qualified health claim petitions that are submitted to FDA will be available for public review and comment. A listing of petitions open for public comment is at the FDA Dockets Management website. A summary of the qualified health claims authorized by FDA may be found at its website.

Nutrient Content Claims

Most nutrient content claim regulations apply only to those nutrients or dietary substances that have an established daily value and are expressed as percent DV. Percentage claims for dietary supplements are another category of nutrient content claims used to describe a percentage level of a dietary ingredient for which there is no established DV.

Structure–Function Claims

Statements that address a role of a specific substance in maintaining normal healthy structures or functions of the body are considered to be structure–function claims. Structure–function claims may not explicitly or implicitly link the relationship to a disease or health-related condition.

Structure–function claims on dietary supplements describe the role of a nutrient or dietary ingredient intended to affect normal structure or function in humans—for example, "calcium builds strong bones." In addition, they may characterize the means by which a nutrient or dietary ingredient acts to maintain such structure or function—for example, "fiber maintains bowel regularity" or "antioxidants maintain cell integrity"—or they may describe general well-being from consumption of a nutrient or dietary ingredient. Structure–function claims may also describe a benefit related to a nutrient deficiency disease (e.g., vitamin C and scurvy), as long as the statement also tells how widespread such a disease is in the United States. If a dietary supplement label includes such a claim, it must state in a "disclaimer" that FDA has not evaluated the claim. The disclaimer must also state that the dietary supplement product is not intended to "diagnose, treat, cure or prevent any disease" because only a drug can legally make such a claim.

2 CFSAN's Consumer Adverse Events Reporting System and MedWatch

The CFSAN has developed the Consumer Adverse Events Reporting System (CAERS), which replaces the patchworks of existing adverse event systems that were maintained previously by individual offices within CFSAN. FDA uses the CAERS as a monitoring tool to identify potential public health issues that may be associated with the use of a particular product already in the marketplace. Information gathered in CAERS also assists FDA in the formulation and dissemination of CFSAN's postmarketing policies and procedures. Currently, adverse event reports

from the dietary supplement industry by consumers and health professionals should be submitted to MedWatch. In the future, it is hoped that even better coordination between Poison Control Centers, and information developed by the DOD on adverse events in service members will evolve.

3 FDA Constituent Update

FDA produces updates for constituents that also provide other information. This information is available at http://www.fda.gov/food/newsevents/constituentupdates/default.htm.

C National Center for Complementary and Integrative Health

The NIH's NCCIH sponsors some research on dietary supplements and also provides fact sheets on a number of products, especially those that are being used for the prevention or treatment of disease. The "herbs at a glance" series contains authoritative fact sheets on a number of different herbs and botanicals, including common names, uses, potential side effects, and resources for more information. Visit http://nccih.nih.gov/health/herbsataglance.htm.

CAM on PubMed is a subset of PubMed that offers free access to more than 462,000 citations of journal articles related to CAM research from the NLM's MEDLINE database and other life science journals. Access it at http://nccih.nih.gov/research/camonpubmed.

D National Cancer Institute

The NIH's NCI operates a number of research programs that involve dietary supplements. Units also occasionally produce fact sheets and papers on cancer treatment and prevention measures that include dietary supplements. The NCI's Division of Cancer Prevention and the Division of Cancer Control and Population Sciences develop and maintain a website called the Dietary Assessment Calibration and Validation Register. These resources are a means of keeping the international and health community aware of worldwide calibration/validation studies on dietary assessment methods. The website is accessible at http://appliedresearch.cancer.gov/cgi-bin/dacv/index.pl. It is particularly useful for researchers intending to do studies that involve nutritional assessment.

Healthcare providers who are treating cancer patients may wish to consult the NCI website for information on dietary supplements and other alternative and complementary therapies for cancer patients. This link can be accessed at http://www.cancer.gov/cancertopics/treatment/cam.

Health professionals and patients who are seeking to enroll in clinical trials of dietary supplements or other therapies for cancers or other diseases should consult the federal government's list of registered clinical trials at http://www.clinicaltrials.gov.

E Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services

The Agency for Healthcare Research and Quality works closely with the NIH and other federal agencies to develop systematic evidence-based reviews of the health literature on topics of public health significance. This agency also operates state-of-the-science and consensus conferences on these topics and publishes the deliberations. Several evidence-based reviews and conferences have involved dietary supplements, including MVM supplements, omega-3 fatty acids, ephedra, and ephedrine for weight loss and athletic performance; antioxidants and vitamins C, E, and CoQ10 for cardiovascular disease and cancer; B vitamins and berries for neurodegenerative diseases; and calcium and vitamin D for bone. The web address is http://www.ahrq.gov.

F National Library of Medicine

1 PubMed and MEDLINE (Public Use)

This is a world famous computerized bibliography, which includes biomedical information on dietary supplements; and access is freely available to the public over the web at www.pubmed.gov. ODS and the NLM partnered to create this Dietary Supplement Subset of NLM's PubMed. PubMed provides access to citations from the MEDLINE database and additional life science journals. Also included are links to many full-text articles at journal websites and other related Web resources.

The subset is designed to limit search results to citations from a broad spectrum of dietary supplement literature, including vitamin, mineral, phytochemical, ergogenic, botanical, and herbal supplements in human nutrition and animal models.

The PubMed Dietary Supplement Subset follows the prior International Bibliographic Information on Dietary Supplements database which was in place from 1999 to 2010, which was a collaboration between the two U.S. government agencies—ODS and the USDA's National Agricultural Library; see https://ods.od.nih.gov/Research/PubMed_Dietary_Supplement_Subset.aspx. The subset provides useful articles on dietary supplements.

2 MedlinePlus

MedlinePlus is the NLM's authoritative consumer health website. It includes consumer health information from the NIH, plus links to information from other U.S. government agencies and trusted health information providers, such as the CDC.

G USDA National Agricultural Library/Food and Nutrition Information Center

The Food and Nutrition Information Center compiles and disseminates authoritative bibliographies for laypeople and generalist practitioners on various topics, including dietary supplements, with partial support for these efforts from the ODS at NIH. These are available free of cost at www.nal.usda.gov/fnic.

H Department of Defense

The DOD has conducted many surveys of dietary supplement use, including the U.S. Army, Air Force, and Navy warfighter personnel [42–51]. The survey questionnaires are well designed and may be useful to other researchers studying very active young men and women. The department has also sponsored expert consensus conferences and also reports by the Committee on Military Nutrition, National Academies of Sciences that deal with dietary supplement use in the military [52,53].

I Uniformed Services University

The Consortium for Health and Military Performance (CHAMP), within the Uniformed Services University, provides resources on various health issues for members of the armed forces as well as the general public through their Human Performance Resource Center (HPRC: hprc-online.org). The HPRC was established to collect, organize, and disseminate the most current information available on all aspects of human performance and provides excellent materials for laypeople, particularly information on dietary supplements and performance. CHAMP also hosts Operation Supplement Safety (OPSS), the DOD initiative focused on dietary supplement education through information, modules, and various multimedia resources (hprc-online.org/opss). The OPSS website also provides access to a High Risk Supplement List and a simple algorithm for risk stratifying supplements.

J Canadian Government Resources

The Canadian government's Natural Health Product Ingredients Database (http://webprod5.hc-sc.gc.ca/lnhpd-bdpsnh/index-eng.jsp) includes a display of toxicity restrictions, registry numbers for the chemicals by Chemical Abstracts Service and other registry numbers, herbals, and hyperlinks to the Natural and Non-Prescription Health Products Directorate.

K U.S. Pharmacopoeia

The U.S. Pharmacopoeial Convention (USP) is an independent, science-based public health organization and official public standards-setting authority for all prescription and over-the-counter medicines, dietary supplements, and other healthcare products manufactured and sold in the United States. The standards are legally enforceable for drugs, and a dietary supplement program also exists. Quality standards are determined by a voluntary expert committee, and products that are submitted for evaluation and pass audits are listed on their website; those products that fail the evaluation are not listed. Currently, there are approximately 100 certified products. Consumer information regarding the USP certification program on dietary supplements can be found on its website as well. Occasionally, USP conducts systematic reviews of various supplements, such as amino acids that are used by body builders to purportedly improve performance. The data can be accessed at www.usp.org/usp-nf.

L Academy of Nutrition and Dietetics

1 Position Papers and Other Materials

The Academy of Nutrition and Dietetics (AND) is the professional association for dietitians. It has developed a number of useful position papers, journal articles, and other materials on dietary supplements [54].

2 Evidence Analysis Library

The AND has created an evidence analysis library that provides authoritative evaluation of the evidence on various clinical topics, including some that involve dietary supplements. Members receive access to the library as part of their dues; access to it by others is by subscription. To learn more, access the AND website at http://www.eatright.org.

3 Practice Groups

The Complementary and Alternative Medicine Practice Group of the AND focuses specifically on dietary supplements. This group produces an excellent newsletter, and members also receive free or reduced prices on many professional resources that are useful in assessing dietary supplement intakes.

M Books

Among the useful reference books are the "Physician's Desk Reference for Nonprescription Drugs, Dietary Supplements and Herbs" [55], which covers the full spectrum of nutritional supplements, including vitamins, minerals, amino acids, probiotics, metabolites, hormones, enzymes, and cartilage products. This resource describes precautions, contraindications, side effects, and possible interactions with medications. The "Commission E Monographs" [56] summarizes the German Commission E monographs on various herbal medicines. "Herbs of Commerce" [57] is a comprehensive listing of more than 2000 botanicals that have current and historical uses as therapeutic agents. Botanical synonyms are included so that older botanical names that are no longer accepted can be cross-referenced. Also included are the Ayurvedic names and the Chinese names for more than 500 herbs. The book contains the Latin binomials (Linnaean classification), the standardized common names, the Ayurvedic names, the Pinyin name (simplified Chinese name), and other common names. The "Encyclopedia of Dietary Supplements" [58] reviews many over-the-counter supplements carried in today's nutritional products marketplace and presents peer-reviewed, objective entries that review the most significant scientific research, including basic chemical, preclinical, and clinical studies. Other authoritative sources are also available [59,60].

VI HOW TO REPORT PROBLEMS WITH DIETARY SUPPLEMENT INTAKE

A Food and Drug Administration

The MedWatch program allows healthcare providers to report problems possibly caused by FDA-regulated products such as drugs, medical devices, medical foods, and dietary supplements. The identity of the patient is kept confidential. Reported adverse effects and drug interactions are also posted on the FDA Dietary Supplement Information Page of its website. If a consumer or healthcare provider thinks a patient has suffered a serious harmful effect or illness from a dietary supplement, it can be reported by calling FDA's MedWatch hotline at 1-800-FDA-1088 or through the FDA website http://www.fda.gov/medwatch/report/hcp.htm. Consumers may also report an adverse event or illness they believe to be related to the use of a dietary supplement by calling FDA at 1-800-FDA-1088 or using the website http://www.fda.gov/medwatch/report/consumer/consumer.htm.

B Federal Trade Commission

The Federal Trade Commission (FTC) has authority over the advertising of dietary supplements. The agency can be accessed at http://www.ftc.gov/bcp/edu/microsites/whocares/supplements.shfm. The FTC has issued advertising guidelines for the supplement industry that explain how truth in advertising applies to this industry and the kinds of claims manufacturers can and cannot make. The guidelines, titled "Dietary Supplements: An Advertising Guide for Industry," can be accessed at the FTC website. The FTC can take action against supplement manufacturers that make claims that lack "sound scientific evidence" in their advertising or that they deem false or misleading. FTC consumer protection can be accessed at www.ftc.gov/bcp/index.shtml.

C Poison Control Centers

The American Association of Poison Control Centers operates a hotline for suspected poisonings from drugs or dietary supplements at 1-800-222-1222. The website can be accessed at http://www.aapc.org. The Poison Post, the free quarterly eNewsletter, frequently has articles on herbals and dietary supplements geared to consumers.

CONCLUSIONS

Best practices today include a careful assessment of dietary supplement use by consumers and patients in order to better assess their health effects. Chapter 1, Dietary Assessment Methodology, provides some additional resources for those working on dietary supplement assessment. In some cases, health professionals will find it useful to encourage the use of specific supplements, and in other cases they will not; in all cases, however, use should be documented.

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Chapter 3

Physical and Clinical Assessment of Nutritional Status

Patricia A. Hume[1] and Tim Ackland[2]

[1]*Auckland University of Technology, Auckland, New Zealand,* [2]*The University of Western Australia, Crawley, WA, Australia*

I INTRODUCTION

A Why Assess Body Size, Shape, and Composition for Health?

The assessment of body size, shape, and composition for health has taken on greater significance because of a worrying increase in the global prevalence of obesity and its related morbidity and mortality. The consequences of obesity are manifest in diminished quality of life, and are felt acutely in the associated healthcare costs. Despite considerable advances in research efforts to understand obesity, the implementation of lifestyle change at the population level has largely failed, so that trends we observe today are likely to continue, increasing the global burden of obesity. This reality mandates a number of important research priorities relating to lifestyle and behaviors, as well as many within the domain of body composition itself.

Research priorities include the need to document and understand the tracking and heritability of body fatness, the need to relate fat patterning in childhood with fat patterning in adults, linking changes in the prevalence of obesity in order to predict disease, mortality, and healthcare costs, and quantifying the protective role of exercise on health in an integrated way using a life-span approach. Although it has been recognized that the development of physical fitness is protective over general health, culminating in the term "health-related fitness" [1], we still require a strategic approach to quantify the dose–response of exercise for enhancing such physiological parameters as blood pressure, insulin resistance, and lipid profile, and provide culturally specific exercise advice matched to developmental stage.

B Why Are Standardized Protocols and Valid and Reliable Measures Needed?

Nutrition status is manifest in body size, shape, and composition. Clinicians must have access to valid, reliable, and cost-effective measures that are comfortable and meaningful for the client undergoing assessment. For an assessment test to be of value it must be sufficiently specific to measure the performance variable of interest, but also reliable so as to detect small changes.

The International Society for the Advancement of Kinanthropometry (ISAK) provides protocols for clinic/field physical assessment of body size and shape (girths, waist:hip circumference ratios, breadths, and somatotype) and body composition (skinfolds). The international training and certification scheme promotes accurate and standardized measurement. For example, ISAK Level 1 is designed for measurement of height, weight, five girths (arm relaxed, arm contracted, waist, hips, and calf), eight skinfolds (subscapular, triceps, biceps, iliac crest, supraspinal, abdominal, thigh, and calf), and two breadths (elbow and knee). These measures enable health and growth monitoring and calculation of the somatotype. Landmarks (identifiable skeletal points) identify the exact location of a measurement site, or from which a soft tissue site is located, to ensure greater accuracy and reliability of measurement. The advantages and disadvantages of ISAK surface anthropometry methods need to be understood.

Clinicians should keep up to date with new technologies and protocols such as the new ultrasound international standards for adipose measurement currently being developed. Assessment of body shape using three-dimensional (3D) scanning is becoming popular in

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research situations. An international standard for 3D body shape measurement for clinical practice is currently being developed by the J.E. Lindsay Carter Kinanthropometry Clinic and Archive. Body composition measurement using multicomponent models, dual-energy X-ray absorptiometry (DXA), ultrasound, and magnetic resonance imaging (MRI) are available for clinical assessment. Variations between machines can result in substantial differences in measurements, therefore the implications of types of machines, operator training, and client presentation need to be understood.

The accuracy of body composition measurements must be taken into account when tracking people over time [2]. Assessing body composition in the growing and developing individual is difficult due to a range of issues. Increasing energy expenditure can reduce fat mass (FM) while fat-free mass (FFM) can be maintained. However, the dual metabolic challenges of exercise and growth deplete the same reserve, violating methodological assumptions concerning stability of the FFM and rendering change detection problematic. Even though a range of methods suitable for children is available, including anthropometry and body density, accuracy in predicting FM and FFM can be poorer than assumed. Individual variability is such that the threshold of performance impairment due to chronic depletion of energy reserves will tend to differ between individuals. In this context, the pragmatic solution is to align anthropometric data with those of performance, fatigue, and general health. Full dialogue with the patient/client is needed.

II ASSESSMENT OF BODY SIZE AND SHAPE

A Field Techniques—Anthropometry

Anthropometric measures are used in clinical practice, population screening, and research to assess growth, the prevalence of underweight or overweight, and to estimate disease risk for conditions such as type-2 diabetes. The ISAK protocols should be followed to improve accuracy, therefore correct land marking is required [3].

1 Height

Stretched stature (height) is measured on a stadiometer. The client stands with the heels together and the heels, buttocks, and upper part of the back touching the scale. The clinician aligns the head in the Frankfort plane (technically the lower edge of the eye socket is in the same horizontal plane as the notch superior to the tragus of the ear) and the client takes a deep breath in whilst the clinician applies upward lift through the mastoid processes (base of the skull) ensuring the clients heels remain on the stadiometer base. The stadiometer head board is lowered onto the head ensuring the hair is compressed. A loss of approximately 1% in stature is common over the course of the day; therefore the stretched stature method is used [4].

Measurement of height is necessary to track changes in growth, calculate body mass index (BMI), and waist-to-height ratio. Height should be directly measured using a stadiometer, except for infants, where body length is best measured using a length board [5]. Height begins to decline at approximately 30 years for men and women, and this decline accelerates with age. In one longitudinal series, between the ages of 30 and 80 years, women lost 8 cm and men lost 5 cm [6]. Height decreases as a result of vertebral bone loss as well as thinning of intervertebral disks and weight-bearing cartilage. Height may also decrease due to vertebral compression fractures in the settings of osteoporosis or trauma. Loss of vertebral mass and disk compression may induce kyphosis (curvature with backward convexity of the spine), which will further reduce measured height.

When height cannot be accurately measured, such as in acutely ill or immobilized patients, alternatives include self-reported height, estimated height, or surrogate anthropometric measures (arm span, knee height, and seated height) [6]. For example, knee height is measured with specialized calipers and is performed either in sitting or recumbent positions, making this useful in most ambulatory and hospital settings [7]. Prediction equations for the estimation of height from anthropometric surrogates are applied for specific age, gender, racial, and ethnic groups. Several trials, that directly compared measured height to surrogates, showed mean differences between measured height and self-reported height (0−2 cm), knee height (−0.6 to 4 cm), and arm span (0−7 cm) when the measurement was conducted in ill patients instead of healthy subjects [8]. Self-reported height is less accurate than measured height because men tend to over report and women tend to underreport their height [9]. Estimation of height by visualization of supine patients has been found to overestimate height so patient self-reported height is more accurate [10].

2 Body Mass (Weight)

Body mass (weight) is measured using calibrated scales (e.g., quality load cell electronic, beam-type, bed scales, chair scales, or wheelchair scales). The accuracy of these instruments should be within 50 g. Calibration of scales using calibration weights totaling at least 150 kg should be conducted regularly. The client needs to stand on the center of the scales without support, with the weight distributed evenly on both feet, and with the head/eyes facing forward (not looking down at the feet). Looking down

or moving the body weight center of mass away from the base of support between the feet will result in a different reading than if the correct stance is followed.

To monitor changes in weight over time, use of the same scale is recommended given the variability between scales. When a person cannot be weighed or provide a self-reported weight (often inaccurate), weight may be estimated; this inaccurate practice does improve with experience [10]. Overweight women and men tend to underestimate weight, whereas lower weight men tend to overestimate [9].

Technological advances now allow automatic remote monitoring of home scales via telephone or the Internet. This method has gained popularity for management of chronic diseases such as congestive heart failure and obesity. Involuntary loss of body weight due to illness is associated with increased risk of morbidity and mortality. In patients with cancer who were undergoing chemotherapy, a loss of 5% or more of usual body weight was associated with impaired functional status and significantly decreased median survival compared to patients without weight loss [11]. Changes in weight are commonly due to alterations in body water with conditions such as congestive heart failure, cirrhosis, and renal failure or with treatments for these conditions such as diuretics.

3 Weight for Height (BMI)

Weight for height is more commonly known as BMI. BMI is calculated as body mass divided by height squared (kg/m^2). The use of BMI to assess weight for height in individuals reflects recommendations of the National Institutes of Health and World Health Organization [12]. BMI correlates with body fat for populations, but there remains considerable variation in body composition among individuals at each level of BMI. BMI may be elevated despite relatively low levels of body fat in those with edema or in athletes. The relationship between BMI and body fat differs between sexes, varies among racial and ethnic groups, and changes over the life span. A single BMI classification scheme for the entire adult age range does not reflect the loss of FFM and gain in FM that accompany aging. Older (>65 years) men and women have a higher percentage of body fat compared to younger counterparts with the same BMI. Women have a higher percentage of body fat compared to men of the same BMI [13]. BMI should only be used as a guide for populations. Both low and high BMI correlate with morbidity and mortality, although there is ongoing debate regarding issues such as the magnitude of risk for those with BMI in the overweight range ($25-30\ kg/m^2$) and how age modifies risk for morbidity and mortality. Low levels of BMI are classified as BMI $<17.5\ kg/m^2$.

4 Arm Span

Arm span is the distance from the tip of the middle finger of one hand to the other. The client stands against the wall with feet together, facing the clinician and raises the arms to horizontal position. The heels, buttocks, and upper back, together with dorsal aspects of the arms contact the wall. The client inspires maximally and the arms are stretched maximally while arm span is measured. The easiest way to conduct this measurement is to have a chart strip attached to the wall close to a corner where one finger is placed. A nonpermanent marker pen is used to mark the distance to the other finger.

Arm span for patients who cannot stand can be measured with arms stretched at right angles to the body by using a measuring tape crossing in front of the clavicles. Demi-arm span (the distance from the sternal notch to the tip of the middle finger of one hand) can also be measured and then doubled to calculate arm span.

5 Girths

Girths are measured with steel tapes to eliminate tape stretching as may occur in material or plastic tapes. A flexible steel tape of at least 1.5 m in length calibrated in centimeters with 1 mm gradations is recommended. The tape should be aligned perpendicular to the length of the limb (e.g., upper arm girth, calf), and should be tight enough so there are no large gaps against the skin, but not too tight to cause skin indentation.

Waist girth should be taken at the minimum girth in the horizontal plane. If the client does not have a minimum waist girth, then the measurement is half way between the bottom rib and the hip bones. The client needs to be breathing normally and the measurement is taken at end tidal breath (end of expiration—but not forced expiration).

Hip girth is taken at the maximum posterior protuberance of the gluteal muscles. If the measurement is taken over clothing, then the tape needs to be pulled more firmly to ensure the measurement is representative of the underlying body structures.

Circumferences of the trunk and limbs reflect amounts of underlying FFM and FM. The "Healthy Hearts" longitudinal study of cardiometabolic health in 902 youth aged 6–15 years showed that children with low cardiorespiratory fitness were characterized by larger waist circumference and disproportionate weight gain over a 12-month follow-up period [14].

6 Circumference Ratios (Waist:Hip)

Central distribution of body fat increases risk for type-2 diabetes, metabolic syndrome, hypertension, and coronary heart disease [15]. Measures of central adiposity include a single circumference, or more commonly, a waist-to-hip

ratio (WHR). WHR correlates well with abdominal fat content and is a predictor of coronary heart disease and type-2 diabetes when using a cut-off ratio of 0.5 as an indicator of risk [16]. WHR has been found to be accurate in children and adults, men and women, and across ethnic groups.

7 Bone Breadths

A small sliding caliper is used for biepicondylar humerus (elbow) and biepicondylar femur (knee) breadths. The caliper should have branch lengths of at least 10 cm, an application face width of 1.5 cm, and be accurate to within 0.05 cm. The caliper needs to be held correctly so that the fingers can palpate the bony landmarks and the caliper branches can be placed correctly. The underlying soft tissue must be compressed so that the correct bone breadth is measured.

8 Somatotype

Somatotype is a quantification of the shape and composition of the human body. Somatotype can be calculated from the restricted ISAK profile items, and has useful applications in growth and aging, body image, and in sports profiling. The somatotype reduces a large number of measures or visual observations to a simple, three number rating (Endomorphy-Mesomorphy-Ectomorphy) which is independent of size, age, and gender. Endomorphy represents relative adiposity of a physique, mesomorphy represents relative musculoskeletal robustness of a physique, and ectomorphy represents relative linearity or slenderness of a physique. Rating values and relative meanings for all three components are 0.5 to 2.5 = low, 3.0 to 5.0 = moderate, 5.5 to 7.0 = high, >7.5 above = extremely high. A novel iPad-based application to rapidly assess body image using somatotype comparisons is now available. It simultaneously assesses body fat, muscle, and leanness using realistic quasi-3D images to provide healthcare professionals with an enhanced tool when dealing with body image disorders [17].

B Laboratory Techniques—3D Scanning

The availability of reliable 3D whole-body scanners and software−hardware suites capable of rapid measurement, data extraction, and analysis has the potential to revolutionize surface anthropometry. The main driver behind 3D scanning has been the apparel industry, envisaging the possibility of "garments on demand," tailored to fit each individual perfectly. 3D scanning has parallel applications in human factors, where humanoid "manikins" can be rescaled using measurements extracted from 3D scans and animated to interact with the built environment. These techniques are already being employed in military contexts and in the design of mass transportation and workspaces.

The basics of a 3D scanning system are four columns each with two laser safety (Class 1) cameras, and software to automatically extract measures. Most scanners work by projecting straight lines or grids onto the human body, which are then distorted by the curves and contours of the body. This distortion is captured by the cameras and decoded to infer the 3D shape. A body scan usually consists of several hundred thousand points, each represented by an XYZ coordinate. These points are joined to their near neighbors to form polygonal meshes. The tiny facets formed by the polygonal mesh can be shaded and smoothed, producing a "rendered" body (the metaphor is that of a plasterer smoothing render over a wire frame).

The resulting images vary depending on the accuracy of the scanner. Body posture during scanning is important to ensure accurate measures can be made from the images. Inside the 3D scanning booth, participants are positioned into standard scanning poses and instructed to hold their breath (at end tidal expiration) for the duration of each 10-second scan (Fig. 3.1).

Prior to scanning, landmark sites are located by palpating underlying bony structures and a physical landmark is attached to the located point. In order to be able to extract traditional measurements, the software must be able to identify these landmarks. There are three common land marking systems used in 3D anthropometry: automatic landmark recognition (ALR), where the software identifies landmarks from the scan without human intervention; digital landmark placement (DLP), where landmarks are located on a digital image by identifying surface features; and physical-digital landmark location (PDL), where landmarks are placed physically on the body and these landmarks are then digitally located on the scanned image. ALR has proved to be unacceptably inaccurate, while DLP is often difficult on fat or very muscular subjects where underlying bony landmarks are hard to locate. Therefore, we rely heavily on PDL, which has the disadvantage of requiring more time and operator skill. There is no universally accepted protocol for 3D scanning; however, protocols are being lodged in the J.E. Lindsay Carter Anthropometry Archive [18].

Three-dimensional analysis can be extended to calculate segmental and whole-body volumes and hence, to estimate body fat percentage. If we know the mass of the subject and the volume estimated by 3D scanning, we can calculate the whole-body density and hence (with certain assumptions regarding tissue density), to estimate body fat percentage. Studies examining the accuracy and precision of using 3D scans to predict whole-body density measured against a criterion standard such as DXA or hydrostatic weighing are needed.

Body changes due to growth, maturation, dietary, and training interventions can be assessed. 3D scanning can be used to visualize and better quantify size and shape

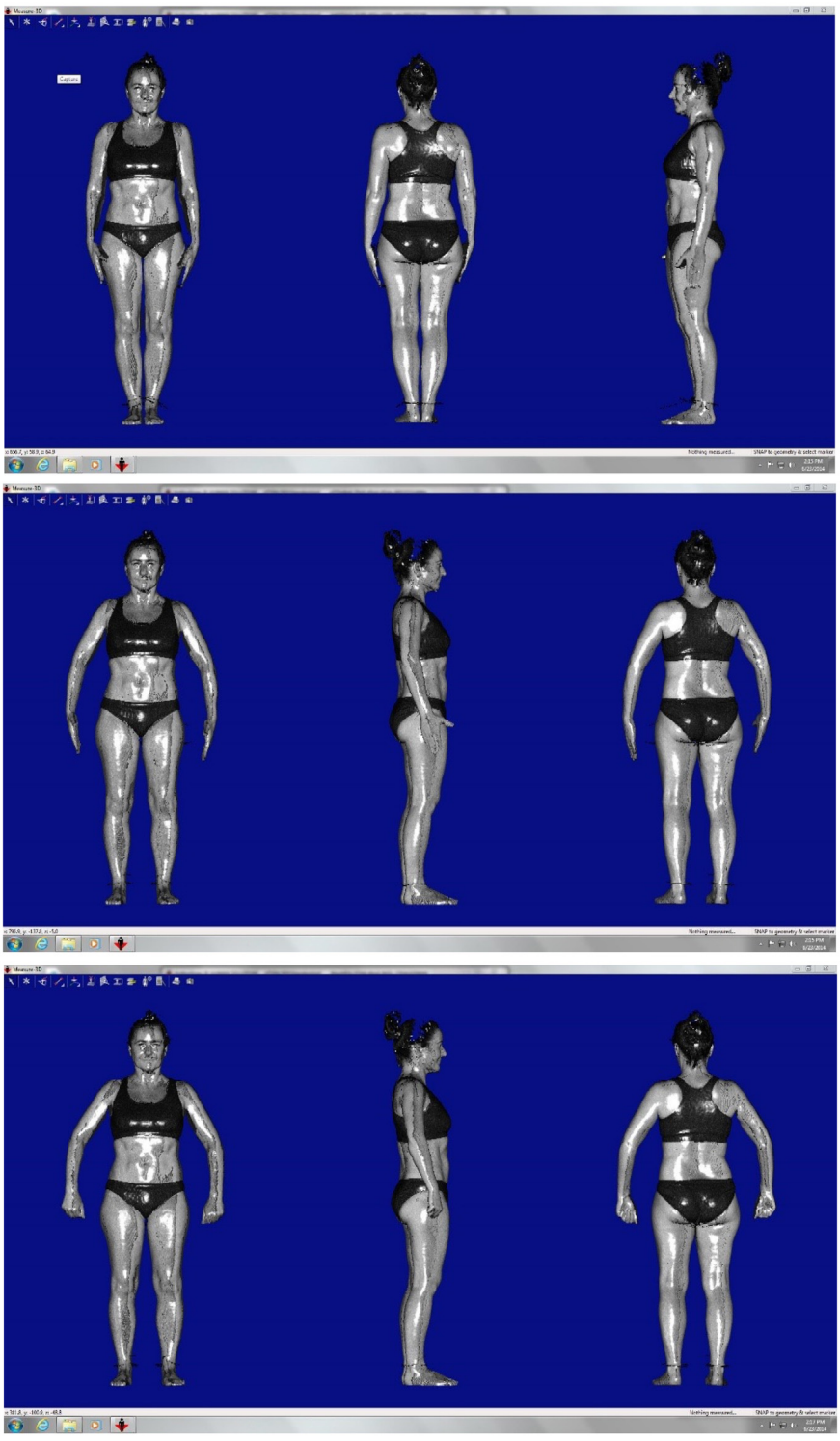

FIGURE 3.1 Example postures for 3D body scanning.

changes that occur due to aging and exercise or nutrition interventions. Somatotyping using 3D anthropometry has been conducted [19], and applications to health and exercise science have been reported [20].

III ASSESSMENT OF BODY COMPOSITION

Quantifying human body composition has been prominent in medical research and allied health clinical practice for many decades. While progress has been significant in recent years, with new and combined analytical methods, various ethical and methodological limitations still prevent the identification of an absolute measurement standard for use in humans. As a consequence, much of the research to validate new and old methods is indirect, and despite considerable advances in methods, today there is still no gold standard for body fat assessment with accuracy better than 1%.

Estimation of body fat has been the prime focus of attention, but many scientists and practitioners recognize that the amount and distribution of lean tissues like bone and muscle can be just as important in determining an individual's health status. Making sense of the myriad techniques for estimating each tissue component requires a clear framework by which these may be properly compared. When monitoring body composition, scientists and practitioners are often limited by assessment time and complexity, as well as equipment cost, ease of use, and portability. These factors conspire against the desire for accurate measurements, with the inevitable consequence that captured data may be misleading, misinterpreted, or perhaps used inappropriately. This reality has forced many to consider acceptable surrogates for fatness, without recourse to quantifying tissue mass.

The discussion in this section will concentrate only on body composition techniques, both field and laboratory-based, that were supported in a recently published Position Statement under the auspices of the International Olympic Committee's Medical and Scientific Commission [21]. The review paper provides a critique of all commonly employed body composition techniques with special consideration of the assumptions, cautions, advantages, and disadvantages of each methodology.

A Field Techniques

Field techniques—those which are portable and allow data collection away from the laboratory—including skinfolds, bioelectrical impedance analysis (BIA), and several relative weight indices (including BMI) have recently been reviewed [21]. Due to the overwhelming limitations and unsubstantiated assumptions associated with the latter techniques, only the use of skinfolds was supported for assessing and monitoring the thickness of subcutaneous adipose tissue (SAT) (as a surrogate measure of body fatness).

1 Skinfolds

Skinfold thickness describes the amount of subcutaneous fat when the fold is lifted and its thickness measured by specialized calipers. The sum of skinfolds (generally from eight sites in the standard ISAK protocol) provides data for comparison with population norms, or for monitoring changes over time within the same individual.

Devices to measure the thickness of a compressed, double layer of skin plus the underlying SAT have been used ubiquitously for well over 50 years. Unfortunately, much of the published data cannot be relied upon due to vast differences in caliper specifications, the number and location of skinfold sites, and a lack of standardization in operator technique and data treatment. There are over 100 body fat prediction equations derived from skinfold measurements [1] and their inconsistent results stem from differences in the populations that were sampled.

The ISAK manual contains details of standardized protocols [3] to help minimize the technical error of measurement for repeat skinfold measures at various measurement sites. The standard ISAK protocol for skinfolds has the following requirements:

- Use approved skinfold caliper that is regularly calibrated. Skinfold caliper requires a constant closing compression of 10 g/mm^2 throughout the range of measurements. They should be calibrated to at least 40 mm in 0.2 mm divisions.
- Precise marking of eight standard measurement sites on the right side of the body.
- Though more skinfold sites are supported, using these eight sites helps to address both intra- and interindividual differences in subcutaneous fat deposition.
- A standard measurement protocol that prescribes the operator technique and order of measurement, as well as providing for a minimum of two (but preferably three) repeated measures at each site.

The importance of accurate skinfold measurement site location has been examined in 12 healthy participants. Nine measurements, in a one-centimeter grid pattern, centered on each of eight ISAK-specified skinfold sites, were taken three times at each grid point by each of two ISAK Criterion (Level 4) measurers using Harpenden skinfold caliper. Skinfolds taken at the eight peripheral grid points in a 1 cm grid pattern were generally different (45 out of 64 = 70%) from the skinfolds taken at a central ISAK grid point. There was also an effect by direction (anterior, posterior, superior, or inferior). The subscapular was the most robust skinfold site with small measurement deviation away from the central ISAK point. All other skinfold

sites showed some variation, with most care needed in marking the biceps and triceps skinfold sites. Measuring 1 cm away from a defined ISAK site produced significant differences in the majority of skinfold measurement values obtained and no site was totally free from this variation. Therefore, adherence to identifying, marking, and measuring at the defined site is essential [2].

Despite the benefits accrued by this standardized technique, there are several unresolved assumptions and limitations of the skinfold methodology [21], that:

- there exists consistency in adipose tissue deposition (fat patterning);
- a fixed relationship of subcutaneous to internal fat exists;
- skinfolds have a constant compressibility;
- the skin contributes to a constant fraction of the skinfold value;
- the lipid comprises a constant fraction of the adipose tissue; and
- water comprises a constant fraction of the adipose tissue.

Furthermore, several studies have shown that skinfold thickness is influenced by age, sex, race, and the state of hydration.

Another important consideration relates to predicting body composition from surface anthropometry. ISAK supports the reporting of individual values as well as a simple arithmetic sum of eight skinfolds when monitoring participants over time, or when comparing individual results to normative data. This reporting standard minimizes the aggregation of assumptions and limitations associated with the use of regression equations for predicting a body fat percentage (%BF). All body fat regression equations use several raw skinfold values to predict a %BF. The equations are generally derived using another surrogate measure of %BF, such as hydrostatic weighing, air displacement plethysmography (ADP), or bioelectric impedance (BIA), as the dependent variable. However, since these supposed "reference" measures are neither valid nor reliable, the value of such regression equations is highly questionable.

B Laboratory Techniques

Several established and emerging laboratory techniques are widely supported [21], including multicomponent models, DXA, and the use of medical diagnostic devices such as ultrasound and MRI. Other common techniques such as densitometry (via hydrostatic weighing, ADP, and 3D scanning) and hydrometry (e.g., total body water) have been employed extensively in research and clinical settings, but these were not supported due to myriad limitations and unassailable assumptions.

1 Multicomponent Models

Elaborate 3-, 4-, 5-, and 6-component models are available for body fat estimation [22]. Their precision and accuracy are in the order of 1−2%. The 4-component model employs separate techniques to estimate body density, body water, and bone mineral, and is the leading method in current use. The equation is in the form:

$$FM = C_1 \cdot BV - C_2 \cdot TBW + C_3 \cdot M - C_4BM$$

where FM is fat mass, C_n is a constant, BV is body volume, TBW is total body water, M is bone mineral, and BM is body mass.

Precision of multicomponent models is high [22], and technical errors of estimating body volume, body water, and bone mineral have been combined to yield a %BF error of approximately 1%.

The multicomponent models are considered the most accurate means of estimating body composition to date, since they accommodate variability in bone mineral and water content; both an issue that invalidates the general two component model. However, this technique assumes a constant proportion of protein to water and that the density of each tissue component is constant. Furthermore, it involves expensive technology, a long analysis process, and its utility suffers for a lack of normative data.

2 Dual-Energy X-ray Absorptiometry

DXA scanners were originally designed to measure bone mineral content and density, though software developments during the 1990s also permitted the estimation of soft tissue masses. This is achieved by passing filtered X-ray beams at two photon energies through the body that are attenuated differentially by the material in their path. The synthesized data map provides the mass and composition of each pixel in terms of bone mineral, FM, and FFM. This chemical assessment of the body should not be confused with an anatomical (tissue) compartment approach. Common interpretation errors include that the FM equates to adipose tissue, that the bone mineral equates to skeletal tissue, and that the FFM equates to muscle. In fact, the FFM aggregates all remaining tissue components that are not fat (lipid) or bone mineral (calcium carbonate and calcium phosphate).

The whole-body DXA scan delivers a low dose of radiation (equivalent to a long-haul plane journey) which varies according to scanner type and beam configuration (i.e., pencil or fan beam). Consequently, this method is not suitable for pregnant women, or for multiple scanning of individuals over time. Clients should not be measured more than four times per year, due to the cumulative radiation dose, and also the error of measurement which limits the ability to detect small changes in body composition over time [21]. Early DXA machines were quite limited

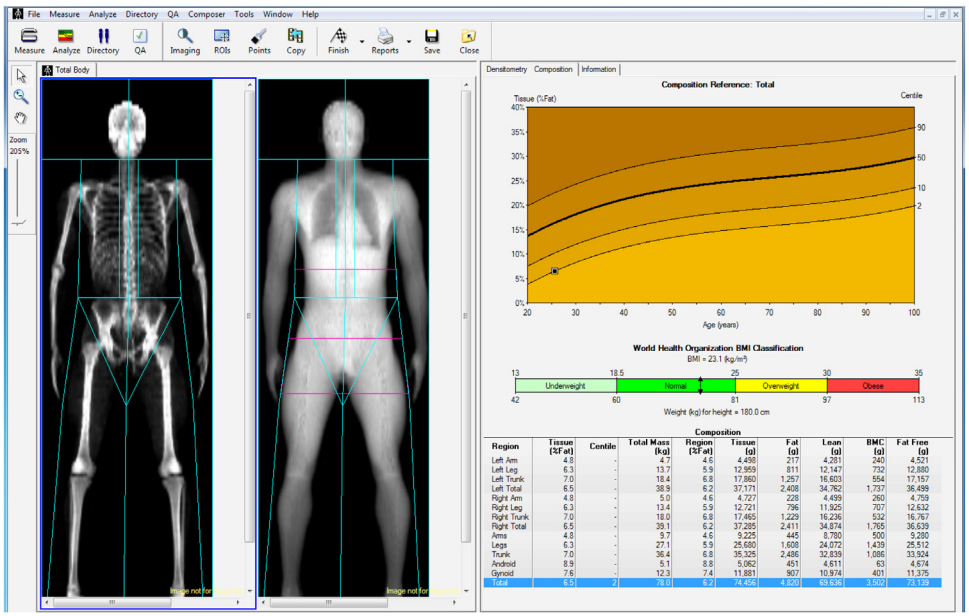

FIGURE 3.2 Whole-body scan report for a lean male participant aged 25 years using a Lunar Prodigy DXA machine (GE Medical Systems). With an overall 6.2% body fat, this individual has 4.82 kg of fat, 3.50 kg of bone mineral, and 69.64 kg of lean tissue.

in the dimensions of the scan bed which presented a problem for whole-body assessments. While more recent models can accommodate clients up to 120 kg mass, most scan beds will not fit clients greater than ~192 cm in length (Fig. 3.2).

Following a standardized DXA scanning protocol is essential for achieving accurate and repeatable measures. One study [23] scanned young physically active participants on five occasions within a 2-day period. The first scan was in the morning after an overnight fast, then approximately 5 minutes later after repositioning the participant on the scanning bed. The next scan followed after an 8-hour period of normal daily activities, followed by a fourth scan the next morning, and a final measurement after breakfast 30 minutes later. There was a negligible effect of participant repositioning; however, daily activities and the consumption of food produced a substantial increase in the typical error and mean values of lean mass and total body mass. Taking these results into account, recommended standard protocols for DXA scanning require clients to be fasted (approximately 12 hours), well-hydrated, and with bowel and bladder recently voided.

DXA-derived body composition estimates have been validated against multicomponent models [24] with SEE (standard error of the estimate) for predicting %BF reported between 2% and 3%. Several assumptions are made in the soft tissue estimates from DXA including beam hardening due to the depth of tissues, magnification errors, and errors of estimating fat quantity in approximately 40% of scan pixels that contain bone [25].

In summary, DXA provides a reasonably precise whole-body method for body composition assessment for most individuals, though it is not reliable in producing accurate fat estimates in very lean individuals (due to the high proportion of pixels containing bone). Differences between machine manufacturers, beam configurations, and software algorithms prevent simple comparison of data that have been derived from different apparatus.

3 Magnetic Resonance Imaging

Though costly and time consuming, medical imaging techniques offer an opportunity to account for both subcutaneous and deep adipose tissue depots, as well as providing estimates for other tissues such as bone, muscle, and viscera. No ionizing radiation is involved, so the method is noninvasive, though the confined space of the scanner may induce claustrophobia.

Whole-body MRI scans are possible and these must be acquired as an integrated series of stacks. The pixel size within slices of 2×2 mm for whole-body scans does limit the accuracy of measurement for lean individuals, while difficulty in discriminating boundaries between tissue layers, further limits sensitivity. MRI produces 3D tissue volumes which require assumptions to be made about tissue density and chemical composition before tissue masses can be estimated. As a consequence of these limitations, medical imaging technologies are not considered to be practical for body composition assessment and monitoring at this point in time. Future advances in hardware

and software, however, may improve the utility of MRI for body composition assessment.

4 Ultrasound

A novel ultrasound measurement and analysis method [26] allows quantification of SAT layers without compression and with high measurement accuracy. For example, a comparison of ultrasound SAT measurements in excised pig tissue with Vernier caliper measurements of 0.1 mm resolution resulted in a very high correlation ($r = 0.998$; $n = 140$) between scores with an SEE of 0.21 mm [27].

The standard eight ultrasound site locations [26] are defined relative to the standing stature of the client for upper abdomen (UA), lower abdomen, erector spine, distal triceps, brachioradialis, lateral thigh, front thigh, and medial calf. These sites help account for interindividual differences in SAT patterning and allow a sum of eight SAT thicknesses to ensure high accuracy and reliability. The sites are marked on the skin with the participant standing or seated, while ultrasound images are captured with the participant lying on a plinth in prone (Fig. 3.3), supine or side-lying postures. Any B-mode ultrasound machine can be used to capture an image for analysis, provided a linear transducer of 5−20 MHz is used. The transducer must have a thick (3−5 mm) layer of gel applied which allows contact with the skin but without compression of the underlying tissues.

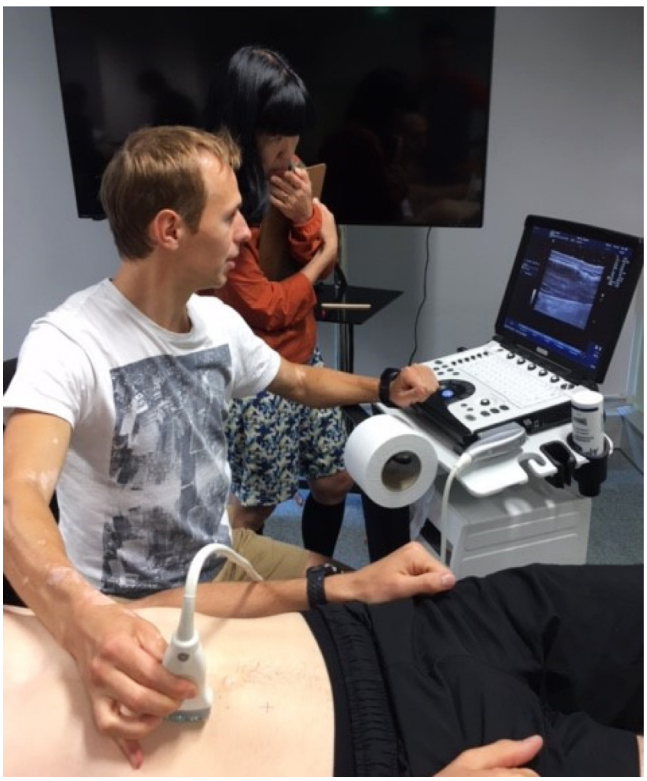

FIGURE 3.3 Dr. Andrius Ramonas (New Zealand) and Dr. Kazuko Ishikawa-Takata (Japan) using an ultrasound to capture an image at the UA site during an ultrasound body composition training course at the J.E. Lindsay Carter Kinanthropometry Clinic in Auckland, New Zealand.

All sites overlie muscle with a clearly visible fascia enabling clear acquisition of ultrasound images and the SAT layers show little variation in thickness in the vicinity of selected site landmarks. Fibrous structures embedded in the SAT, such as the Camper's fascia in the abdominal sites, can be quantified using the semiautomatic image evaluation software [26]. Visual control of the FAT measurement algorithm eliminates errors associated with automatic contour detection algorithms. The routine measurement of many thickness values in a single image, the possibility to include or exclude embedded structures like fibrous tissues and blood vessels in these thickness values, and the ability to quantify their depth is greatly advantageous for the scientist or clinician.

With training and practice, the land marking, image capture, and evaluation of eight sites takes about 20 minutes per individual. This technique has the potential to fill a void in our ability to measure and monitor body fat with accuracy and repeatability using a relatively inexpensive technology. While assumptions are necessarily made about the relationship between SAT and internal adipose tissue depots, this methodology removes several of the limitations attributed to skinfolds. Published reference data using this ultrasound technique for a variety of subpopulations are now needed so that the methodology may reach its full utility.

IV CLINICAL CONSIDERATIONS IN ASSESSMENT OF NUTRITION STATUS

A Consent to Conduct Measurements

Body measurement can be stressful for patients/clients, so a clear explanation of the measurement process should be outlined. It is recommended that an information sheet be provided to the client explaining what will be happening to them, what they should wear, what measurements will be taken, by whom, and approximately how long the measurements will take. Written and verbal informed consent expressed in plain language should be obtained from every client, or from their legal parent or guardian, if they are minors or are incapable of making or communicating an informed decision.

B Understanding Growth Changes

Body composition changes during childhood, adolescence, and adulthood impact health and physical performance. By understanding the biology of growth we can

anticipate adult morphology, and relate this to optimal skeletal size and proportions (morphological optimization), and change in soft tissue for maximum functional effectiveness (morphological prototype). Proportionality is important for the changing physical parameters during growth. Segment breadths are most useful for predictive purposes because they remain stable in relation to stature throughout adolescence. However, there is nonuniformity of the tempo and timing of body tissues and systems undergoing growth. Recognition of the wide individual variability in maturation rate by clinicians helps interpretation of data and the caution needed for database normative comparison values.

Normal growth can be undermined by nutritional deficiency, impairment of hormone production, or both. Growth hormone facilitates protein synthesis, and mediates the proliferation of cartilage cells at the epiphyseal boundary which promotes somatic growth. Thyroid hormone promotes growth of the central nervous system and works in conjunction with growth hormone to stimulate bone and cartilage formation, while insulin-like growth factor is influential in protein synthesis and muscle growth. Puberty commences with increasing complexity and interaction of hormonal influence (growth hormone, insulin-like growth factor, thyroid hormone and cortisol, plus testosterone in boys and estrogen in girls). Hormones largely determine the tissue mass gained during adolescence—mainly muscle in boys and fat (and also muscle) in girls.

C Tracking Somatic Growth Through the Life Span

Anthropometric measurements allow comparison to population norms or to values collected over time in the same individual. Several markers of somatic growth are typically used to track children to adolescence. Stature and mass velocity curves rapidly decelerate in early childhood until three years of age when stature deceleration is reduced and the rate of weight gain begins to increase. However, due to the inability of younger children to tolerate measurement procedures, assessments of stature and mass (and hence BMI) are prone to error, and data need to be interpreted with caution.

The growth rate in infancy exceeds that of the adolescent growth spurt. Less widely recognized is the mid-growth spurt typically occurring between 6.5 and 8.5 years, which is much smaller and less marked. Importantly, the capacity to detect this mid-growth spurt is limited by measurement frequency and measurement precision. Many children might only be measured annually, in which case the mid-growth spurt is unlikely to be detected. Precision error of measurements must be sufficiently small, so the modest effect of this spurt is not masked by the error of measurement. The typical adolescent growth spurt, later and more marked in boys, is more apparent and easily detected. The difference in stature between adult men and women is mostly because boys grow approximately 5 cm per year for two extra years before commencing their growth spurt.

Peak height velocity typically displays a 2-year difference between boys and girls. Since the 1950s there has been a secular trend for an earlier peak height velocity in both boys and girls. The gain in body mass also exhibits a similar profile, with males showing the same time lag, relative to females. However, different body tissues experience differential growth. For example, an average healthy boy of 14 years might have achieved 91% of his adult stature, but only 72% of his adult muscle mass, and only by 18 years will muscle mass rise to 91%, when stature growth might be approaching zero [28]. The primary tissues of the skeletal system, muscular system as well as adipose tissue all follow a trajectory in adulthood which is a function of the interactions between genetic, hormonal, and environmental factors.

D Body Fat Changes Through the Life Span

Alteration in the relative quantities of fat during childhood is viewed as a health risk factor later in life. While BMI is inadequate for assessing body composition on an individual basis, there appears to be evidence of a link to disease in population studies.

The assessment of body composition in children has taken on greater significance because of the likelihood of obese children becoming obese adults, and that lifestyle behaviors acquired in childhood are resistant to change. While our understanding of etiology of childhood obesity remains incomplete, several factors have a direct or indirect influence over energy intake and energy expenditure. Higher body fat content is associated with early maturation in girls (early menarche) and greater fat acquisition throughout adolescence. The effect of body fat content on maturation is less well established in boys, possibly as a result of the rapid increase in muscle mass at this time. Earlier sexual maturation in girls, relative to boys, means that physical activity is de-emphasized, commonly in favor of social activity. While physical performance capability of boys may exceed that of girls, the importance of maintaining physical activity in terms of health, can be argued to be of greater importance for girls. Increasing energy expenditure via exercise can help reduce fat while FFM is maintained. However, assessment of change in FM and FFM needs to take into account the instability of the latter during the growth and developmental processes [29], which is rarely appreciated.

Adipose tissue tends to be gained systematically in both males and females during adulthood. Observations of fat patterning, and typically the android and gynecoid shapes attributed to its distribution, are well established. Males accumulate more visceral fat than women and tend to accumulate fat on the torso, while females accumulate fat in the gluto-femoral region. Gluto-femoral deposition of fat appears to be protective of health if it offers an alternative depot for excess fat. After menopause, females show signs of increased centralization of excess fat to the torso, including both the subcutaneous and visceral depots.

E Skeletal Size, Shape, and Bone Mineral Density

Bone mineral gain during adolescence is greater than the subsequent loss in later life after peaking around 30 years of age. Because up to 40% of the variation in peak bone mass is considered to be modifiable by environmental factors, exposure to adequate high impact exercise and calcium intake during adolescence is essential to acquire a high peak value, and safeguard against osteopenia and osteoporosis in later life.

Skeletal size and shape are configured earlier in the growth trajectory than bone density. After peak height velocity is attained in the adolescent growth spurt, stature declines to zero growth, although incremental growth in skeletal breadth (e.g., biacromial breadth and bicristal breadth) and chest depth commonly continue after the adolescent growth spurt. Final stature does not indicate that the skeletal shape or peak bone density are finalized. Significant height (commonly up to 8 cm) may be lost in old age as a result of postural changes and structural alterations in the spine caused by osteoporosis. Weight bearing exercise in adulthood generally maintains or reduces the rate of loss of bone, rather than adding bone.

Skeletal age assessment, which is perhaps the most reliable of maturation indices, incurs X-ray exposure and consequent ethical issues. Segment breadths remain relatively stable throughout adolescence and can be used for predictive purposes; however, segment lengths are usually unstable. Segment length variability (e.g., a 6% change in leg length to sitting height ratio) can form the basis for a final stature prediction based on a maturity offset [30].

F Muscle Tissue Changes

Chest depth enlarges as a result of loss of compliance of the thorax. During young adulthood, muscle mass tends to accumulate, however, the extent of accrual depends on activity and interaction between genetic and environmental factors. The widely recognized decline in FFM commencing in adulthood until old age tends to be primarily the loss of muscle tissue, which in turn is related to a decline in growth hormone. Loss of muscle mass has been estimated to be 3 kg per decade on average in sedentary individuals [31], with males losing approximately 1.5 times that of females. Exercise can reduce the rate of muscle loss by about 25% [32]. Muscles in regular use experience less atrophy, and the regeneration of muscle tends to replace slow twitch fibers more than fast twitch fibers, with the effect that slower, less forceful muscle action is facilitated. Elderly individuals undergoing surgery may be unable to restore presurgery levels of the affected muscle mass despite intensive exercise intervention.

G Use of Growth Charts and Normative Data Sets

The scope for body composition change throughout life (childhood, adolescence, and adulthood) from a health perspective is vast. The physique trajectory which has both genetic and environmental determinants can be anticipated up to a point, for identifying disease.

Growth charts have been in routine use since the late 1950s, but are not as straightforward to apply as they might seem [33]. A secular trend for increased stature of between 0.5 and 1.5 cm per decade reported during the 1970s appeared to have ceased by 1980. The validity of this or other secular trends is contingent on the similarity in morphology of the source data to those under investigation, and the sample size reflecting population parameters of ethnic variation and socioeconomic status. Consideration is required of source data for growth charts because cross-sectional growth data, while useful in some respects, cannot illustrate normal growth variation in the timing and duration of the growth spurt. The diagnosis of a growth disorder might be affected by the normative data used for comparison, as well as the measurement technique used, which highlights the importance of standardization of approach and the use of currently validated methods.

We need to be mindful of how to interpret the trajectory of body composition change in adulthood. As a person ages, the probability of death increases, and the mean survival age is less than the mean life expectancy in the absence of premature death. The aging population is increasingly highly selected so our interpretation of morphological changes needs to take this into account. The capabilities of modern medicine prolong life and slow disease progression. These factors are important when considering cross-sectional data, which typically form the evidence base for the study of aging.

Secular trends in life expectancy have shown changes in body composition in later stages of life being the

consequence of a health disorder which may at some future point prove fatal. For example, approximately 30% of Americans aged 60−69 years have no chronic health conditions, but this reduces to 10% for adults 80+ years. The shape of curves for body weight, BMI, or total fat, with respect to age, tend to decline in old age, reflecting the enhanced survival of less overweight individuals. The limits of variability of human physique with impaired health are being extended, as evidenced by living adults with body mass below 30 kg in severe undernourishment, and exceeded 600 kg in extreme obesity.

H Exercise and Changes in Body Composition

While muscle tissue obeys the law of specificity, in terms of use and retention, it does not recruit fat stores locally, so the concept of "spot reduction" in fat stores is unfounded. Interventions which reduce overall body fat will tend to reduce all depots of fat, and not target one depot in favor of others. Guidelines for the general population published by the U.S. Department of Agriculture recommend individuals to engage in moderate to vigorous intensity activity on most days per week, while not exceeding caloric requirements. This advice was tested in a 12-month randomized control trial of sedentary individuals [34] with modest, though significant reductions in weight, BMI, waist circumference, and body fat assessed by DXA. There was an effect of exercise preferentially utilizing abdominal fat in previously sedentary individuals. Increased exercise can add muscle mass while at the same time reducing FM, with the result that weight change is minimal. Individuals with only a small reduction in weight can, therefore, be healthier in terms of risk profile via a range of cardiorespiratory, metabolic, and neuromuscular factors, and have greater functional capacity as a result of adaptation to exercise.

Many studies of the efficacy of dietary interventions consider weight loss or change in BMI as the primary outcome variable. This is usually because they are easily measured, and have large data sets available for comparison, even though body composition measurement would be more valid.

I Athletes and Body Composition

An International Olympic Committee Medical Commission working group on Body Composition, Health, and Performance was commissioned in 2010 to investigate how to protect the health of athletes. It identified three categories of sports that represent a health risk due to training and precompetition practices associated with manipulating body mass: gravitational sports, weight category sports, and aesthetic sports. Gravitational sports involve the body working in a vertical plane by applying forces to overcome gravity, such as endurance running, cycling, and ski jumping. Weight category sports include all types of combative sport such as judo and taekwondo, as well as rowing and weightlifting. Aesthetic sports involve appearance, poise, and highly skilled body movements such as figure skating, gymnastics, and diving. These sports share a common influence of the pressure for leanness, minimizing fat levels, and optimizing power to weight ratio. Several important publications resulted from the activity of this working group include:

- identifying medical problems due to unhealthy practices in sport leading to extremes of underweight, weight reduction, and dehydration [35];
- recognizing research needs in body composition, health, and performance [21,26,36];
- identifying the current practice for body composition assessment globally [36];
- developing suggestions for practical strategies to solve body composition and underweight problems in sports [37]; and
- establishing, if practicable, optimum body composition and/or minimum weight values for healthy competition in sports [35].

V CONCLUSION

Nutrition status is manifest in body size, shape, and composition. ISAK protocols should be followed for clinic/field physical assessment of body size and shape (girths, breadths, waist:hip circumference ratios, somatotype) and body composition (skinfolds). The advantages of the ISAK surface anthropometry methods are that assessments take only 10 minutes for a restricted profile and 30 minutes for a full profile, and the equipment is easily calibrated and readily available. The methods have been shown to be valid and reliable if ISAK training is undertaken to ensure correct land marking is performed. International data are available for comparison of client measures. The disadvantage of the ISAK surface anthropometry techniques is that skinfold caliper compresses the adipose tissue resulting in variation in measurements. New methods such as ultrasound enable more accurate measurement of adipose tissue with improved accuracy. Assessment of body shape using 3D scanning is limited due to the expense of the scanners and correct body positioning in the scanner is essential to gain valid measurements. International standards for 3D body shape measurement are still being developed. Body composition measurement using multicomponent models, DXA, MRI, and ultrasound are expensive due to the cost of the technologies; however, they are becoming more available for clinical assessment. Variations between machines can

result in substantial differences in measurements, therefore the type of machine and the operator and client presentation characteristics for the assessment must be noted.

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Chapter 4

Energy Requirement Methodology

James P. DeLany
University of Pittsburgh, Pittsburgh, PA, United States

I INTRODUCTION

Knowledge of energy requirements throughout the life cycle and during various physiological conditions and disease states is essential to the promotion of optimal human health. Unfortunately, available instruments for measurement of energy intake have demonstrated considerable misreporting [1]. Therefore, investigators have focused on measurement of energy expenditure, which can be accurately measured. However, with the application of the intake-balance technique, utilizing longitudinal assessments of total energy expenditure and body composition, accurate assessments of energy intake can be made [2,3]. Surprisingly, many of the same issues that are currently under investigation, such as gender differences, energy requirements of infants, the effect of different diets, caloric restriction, and physical activity, were first studied in the early 1900s [4–12]. The aim of this chapter is to familiarize the reader with current techniques available for measurement of energy expenditure used to estimate energy requirements.

II COMPONENTS OF DAILY ENERGY EXPENDITURE

Total daily energy expenditure (TEE) is the sum of resting metabolic rate (RMR), the thermic effect of food (TEF), and energy expended in physical activity (EEPA; Fig. 4.1). The methodology utilized to determine each component is indicated to the right and will be described in detail later. The pathway of energy production from the oxidation of macronutrients in the human body is depicted in Eq. (4.1):

$$\text{Macronutrients} + O_2 \rightarrow \text{heat} + CO_2 + H_2O \qquad (4.1)$$

Examination of this equation indicates that to estimate energy expenditure, one could measure macronutrient or oxygen consumption, or the production of heat or carbon dioxide. Most energy expenditure methods in use today rely on measurement of carbon dioxide production and/or oxygen consumption. Measurement of heat production, or directly calorimetry, is rarely used today.

A Resting Energy Expenditure

Resting metabolic rate (RMR), also called resting energy expenditure (REE), the largest component of TEE (Fig. 4.1), is the energy expended by a fasting individual at rest in a thermoneutral environment. The term basal metabolic rate (BMR) or basal energy expenditure (BEE), although often used to describe this component of energy expenditure, is not identical to RMR. By definition, BMR measurements are made early in the morning, in a thermoneutral environment, before the individual has engaged in any physical activity, and with no ingestion of food, tea, or coffee, or inhalation of nicotine-containing tobacco smoke for at least 12 hours before the measurement. If any of the conditions for BMR are not met, the energy expenditure should be termed the RMR. For practical reasons, BMR is rarely measured. In its place, RMR is used, which is generally higher than BMR. To estimate energy requirements, RMR is often measured or estimated based on standard equations and multiplied by a factor to account for physical activity.

1 Determinants of REE

The determinants of REE are well established in both adults and children. The principal factors contributing to individual variation in REE include body size and composition, gender, race, age, physical fitness, hormonal status, genetics, and environmental influences [13–19].

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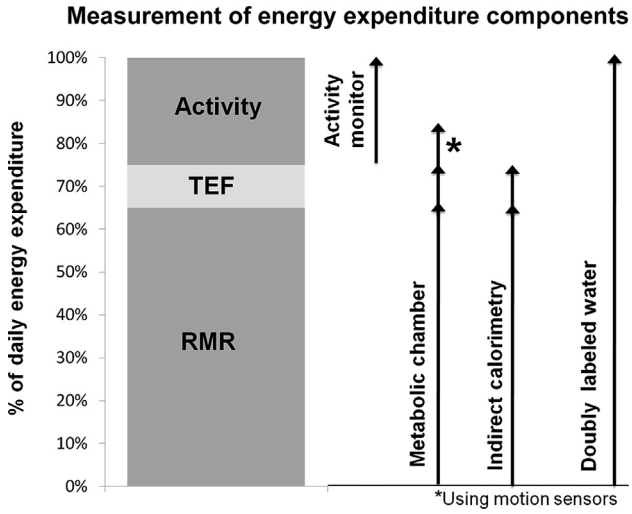

FIGURE 4.1 The components of total energy expenditure.

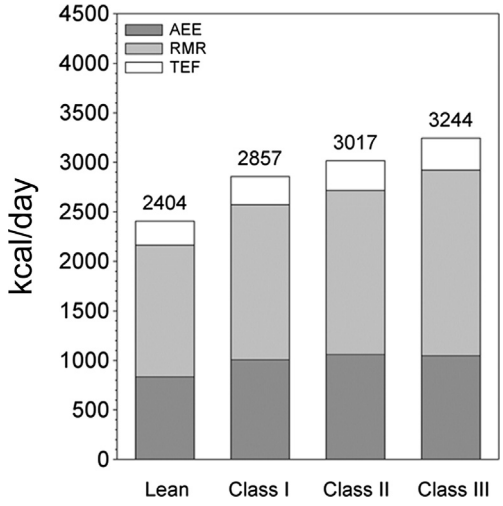

FIGURE 4.2 Increased energy expenditure with increased body size.

a Body Size

Larger people have higher energy requirements than do people of smaller size because additional body tissue requires additional metabolic activity. A recent study in which energy expenditure was measured in lean, Class I, Class II, and Class III obese individuals, with mean body weight from 63 to 124 kg, illustrates the increase in RMR with increasing body size (Fig. 4.2) [20]. Although REE is higher in larger individuals, many factors beyond body weight, such as proportion of body fat, tissue growth or repair, environment, race, and genetics, can induce major differences in metabolic rate.

b Body Composition

The composition of the body has a major effect on REE. FFM, which serves as a surrogate for the metabolically active tissue in the body, is the primary determinant of REE [14]. Hence, most of the variation in REE between people can be accounted for by the variation in their FFM [16]. Several factors influence the amount and proportion of FFM, including age, gender, and physical fitness. FFM can be accurately measured using a number of techniques, including underwater weighing, measuring total body water using stable isotopes of deuterium or oxygen-18, and dual-energy x-ray absorptiometry (DXA). DXA is a technique that accurately estimates FFM, fat mass, and bone mineral mass. Subjects lie supine on a padded table for 6–12 minutes, during which time two very low-energy x-ray beams are passed through the body (Fig. 4.3). The total x-ray dose is generally 1 mrem or less, on the order of a single day's background radiation for a whole-body composition analysis. The technique differentiates bone from soft tissue (Fig. 4.3, inset) and further differentiates soft tissue between lean and fat. Svendsen et al. [21] discuss in detail the calculation of fat mass and lean body mass.

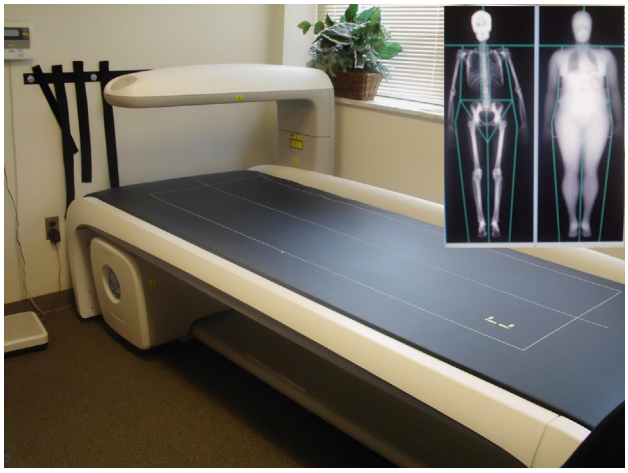

FIGURE 4.3 Dual-energy x-ray absorptiometer for body composition.

Because of the expense or impractical nature of research techniques for body composition, other, less accurate methods such as anthropometry and bioelectrical impedance are often used in practice to estimate body composition. However, in 1999, the National Health and Nutrition Examination Survey (NHANES) began performing DXA body composition measurements on survey subjects 8 years old or older in three mobile examination centers [22].

c Gender

Since men are generally bigger than females, and females typically have more fat in proportion to muscle than males, RMR is generally higher in males than in females. In the data set presented in Fig. 4.2, RMR was higher in

men than in women (2122 ± 453 vs 1659 ± 270 kcal/day; $p < 0.0001$). However, when adjusting for FFM, fat mass, age, and race, RMR was similar in men and women (1690 ± 58 vs 1720 ± 13 kcal/day; $p = 0.61$). Other investigators have also found similar RMR in men and women when adjusting for body composition differences [23,24], although gender differences have been observed in older adults [24,25].

d Age

There is a well-documented age-related reduction in RMR [26–29], with the decline occurring at approximately age 40 years in men and 50 years in women [26]. This decline in REE can be partly explained by a reduction in the quantity as well as the metabolic activity of lean body mass [30], including changes in the relative size of organs and tissues [31]. If individuals gain weight as they age, RMR may actually increase because of gains of FFM and fat mass.

e Physical Fitness and Activity

Athletes generally have a higher RMR compared to nonathletic individuals [32,33]. Whether physical activity has a direct effect on RMR beyond changes in body composition is unclear [19,34,35]. Nonetheless, aerobic exercise and strength training have been shown to result in significantly higher metabolic rates in men and women as well as lean and obese individuals [34–37].

f Hormonal Status

Hormonal status can impact metabolic rate, particularly in endocrine disorders affecting thyroid hormone status. Stimulation of the sympathetic nervous system, such as occurs during emotional excitement or stress, increases cellular activity by the release of epinephrine, which acts directly to promote glycogenolysis. There appear to be no major effects on RMR from the hormonal changes that occur during the menstrual cycle in African-American or Caucasian women [38–41]. Other hormones, such as cortisol, growth hormone, and insulin, can also influence metabolic rate [42–44].

Although leptin was initially considered to be the long-sought antiobesity hormone, leptin treatment in patients with common obesity has not been effective, although it may be useful in conditions such as lipodystrophy [45–50]. Some reports had suggested that a decrease in leptin levels may be responsible for the decrease in RMR observed with weight loss [51–53], but others have found that fasting leptin levels are not independently associated with the decrease in RMR [54,55]. However, recent data support a strong role for leptin signaling in energy expenditure and satiation in the weight-reduced state [56].

g Ethnicity and Genetics

Ethnic origin and genetic inheritance have been shown to affect RMR. Numerous studies have reported that RMR is lower in African-American adults and children compared to non-Hispanic whites even after appropriately adjusting for differences in body composition [39,57–64]. At least some of this difference may be due to the mass of highly metabolically active organs in the trunk region, which is often estimated as the FFM in the trunk region, which have been shown to be associated with the lower RMR in African-American women [57,65,66]. However, in at least one study, the racial difference in RMR was not explained by the FFM in the trunk [67]. However, in this study, the investigators found that European ancestry in African-Americans was strongly associated with a higher RMR, suggesting that population differences in RMR may be due to genetic variation. Another potential explanation for the lower RMR in African-Americans may come from observations of mitochondrial DNA haplogroups. Common African mitochondrial DNA haplogroups have been shown to be associated with lower RMR [68].

A lower RMR has also been reported in Polynesian women compared with Caucasian women after adjusting for FFM and fat mass [69]. No significant differences have been observed in RMR in other ethnic groups investigated. For example, in Pima Indians, a group believed to have a form of genetic obesity, neither RMR nor sleeping metabolic rate have been found to differ from that of non-Hispanic whites after adjustment for body composition [15,70]. Mohawk Indian children were reported to have higher values of TEE than those of non-Hispanic white children, but the difference was due to higher levels of EEPA [71].

Genetic inheritance that determines body composition has a major effect on RMR, accounting for 25–50% of interindividual variability [72]. There also appears to be genetic influence beyond body composition because a significant intrafamily influence on RMR independent of FFM, age, and gender has been reported [17]. Understanding the genetic determinants of human obesity is particularly challenging because common forms of human obesity are largely polygenic. A recent genome-wide association study and meta-analysis of BMI in up to 339,224 individuals revealed 97 BMI-associated loci, 56 of which were novel [73]. The 97 loci account for 2.7% of BMI variation and genome-wide estimates suggested that common variation accounts for >20% of BMI variation. For example, a variant of kinase suppressor of Ras 2, originally identified in a large-scale screen for obese phenotypes in mice, and recently identified as a human adiposity gene, is associated with an RMR that is ~180 kcal/day lower than in those without the variant [74].

Because currently identified genetic variants do not fully explain the heritability of obesity, other forms of variation, such as epigenetic marks, have been considered [75]. Epigenetic marks, which affect gene expression without changing the DNA sequence, have been shown to cause extreme forms of obesity such as Prader–Willi syndrome, but the epigenetic contribution to common forms of obesity is unknown.

h Environmental Influences

The effects of environmental temperature on RMR are conflicting. Well-controlled studies of the acute effect of heat and cold demonstrate significant elevations in metabolic rate [76–79]. However, results from longitudinal studies of the effect of season (temperature) are conflicting, with findings of no effect, a small effect, or as much as 14% increase in metabolic rate [80–82]. High altitude has also been shown to result in increased RMR [83].

i Weight Loss and Weight Gain

A potential barrier to sustaining weight loss is that as individuals lose weight, metabolic adaptation, or a reduction in energy expenditure is observed beyond that expected due to loss of FFM and fat mass [63,84–86]. One question that had been raised is whether this reduction in RMR could be blunted by the addition of physical activity. However, added physical activity has not been effective in blunting the decrease in RMR [55,87]. For example, RMR decreased by 356 kcal/day after 15 kg weight loss in response to 6 weeks of intensive intervention, including supervised physical activity in severely obese individuals [55]. Weight loss of 58 kg at 30 weeks resulted in a decrease in RMR of 851 kcal/day.

Another question about weight-loss-induced metabolic adaptation is whether it persists over time. However, long-term studies have shown that the reduced RMR persists. The reduction in RMR in individuals who lost at least a 10% body weight was shown to persist for >1 year [88]. A longer-term follow-up in the participants who lost 58 kg described above also showed that the lower RMR persists [89]. After 6 years, even though participants regained 41 kg of the initial weight loss, RMR was 704 kcal/day lower than at baseline. The fact that in response a 41 kg gain in body weight gain, there was no increase in RMR (1903 kcal/day vs 1996 kcal/day after 58 kg weight loss) was surprising. The observation of sustained metabolic adaptation even after weight regain is consistent with findings from the classic Minnesota semi-starvation experiment which demonstrated lower RMR during a period of weight regain with controlled feeding [90].

While there are numerous studies examining the decrease in RMR associated with weight loss, there are few reports of the increase in RMR with weight gain. Cross-sectional studies comparing individuals of increasing body weight, such as that presented in Fig. 4.2, indirectly support increased RMR with weight gain. In a study examining the effect of weight gain on energy expenditure, although weight gain was associated with a significantly higher TEE, the increase in RMR of 147 kcal/day in 13 nonobese and 134 kcal/day in 11 obese individuals, was not significant [85]. However, several short-term overfeeding studies have demonstrated an increase in RMR in response to weight gain. For example, a body weight gain of 6 kg induced by 42 days of overfeeding in lean young men was associated with a 12% increase in metabolic rate at rest [91]. Another 42-day overfeeding study in lean and obese men resulting in a weight gain of 7.6 kg was associated with a 12% increase in RMR [92]. In response to 100 days of overfeeding resulting in 8.1 kg of body weight gain, RMR increased by 9.7% [93]. Not taking into account the increase in RMR that occurs in response to weight gain has led to large underestimates of the energy imbalance gap, or the average daily imbalance between total energy intake and total energy expenditure responsible for long-term weight gain [94]. The actual energy flux gap, which is observed with higher energy intake and energy expenditure (e.g., see Fig. 4.2) associated with the higher weight is relatively large [94].

2 Adjustment of REE for Differences in Body Size

In order to properly compare energy expenditure between individuals varying in body size or composition, it is imperative that an appropriate strategy be employed to adjust energy expenditure for these differences. Surface area was used as an early normalizing factor because heat is lost through the skin, so it was assumed that metabolic rate would be proportional to the amount of skin [95,96]. Fat-free mass (FFM) is the normalizing factor used most often today, which is used as a proxy for the metabolically active tissues in the body. The use of these two factors to normalize RMR provides similar results, although higher correlations are generally observed with FFM [97].

In the past, when examining whether there were differences in RMR between groups differing in body weight and composition, some investigators adjusted RMR by dividing by FFM. However, although there is a very strong relationship between RMR and FFM, this relationship has a nonzero intercept, making this practice invalid [98,99]. As an example, in Fig. 4.4, RMR is plotted against FFM for 131 lean and obese African-American and Caucasian children [100]. It is fairly clear that even the most obese individuals, those with

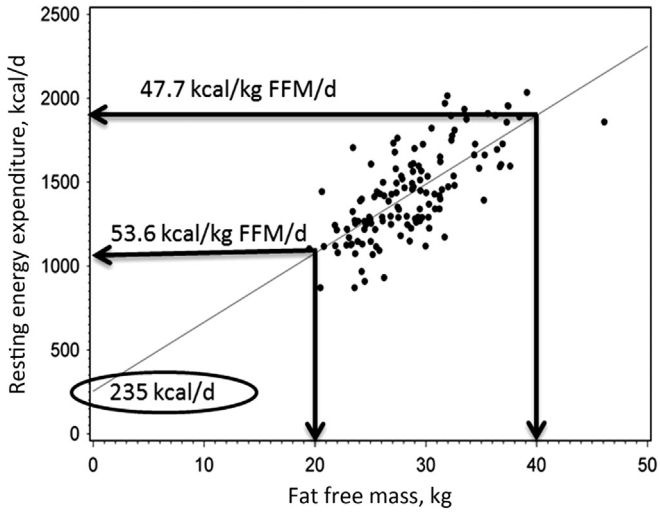

FIGURE 4.4 REE versus FFM.

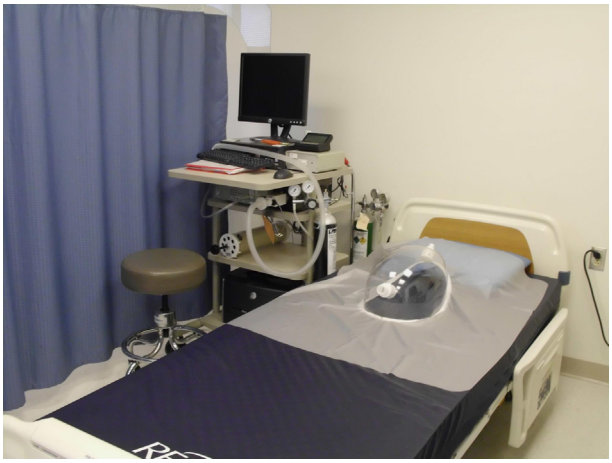

FIGURE 4.5 Indirect calorimetry system for measurement of RMR.

FFM > 30 kg, fall on the regression line for all subjects (RMR (kcal/day) = 41.8 · FFM (kg) + 235; $r = 0.72$). It is also clear that the *y*-intercept is not equal to zero. When comparing a lean individual with 20 kg of FFM with an obese individual with 40 kg FFM, absolute RMR is of course higher in the obese individual (1907 kcal/day) than in the lean individual (1071 kcal/day). However, if one were to divide RMR by FFM to compare these two individuals, it would incorrectly appear that "adjusted" RMR is 11% lower in the obese individual (53.6 vs 47.7 kcal/kg FFM in the lean and obese individual), when in reality these two individuals fall on the same regression line and hence have normal RMR based on body size. In fact, the obese children in this study had a higher RMR when adjusted for FFM but similar RMR when adjusted for FFM and fat mass. Appropriate strategies to adjust for differences in body size are to include FFM as a covariate [20] or to utilize regression methods to adjust for FFM [98]. Other factors that have been included to improve energy expenditure prediction equations include age, race, fat mass, and sex [20,98,101].

3 Measuring REE: Indirect Calorimetry

Metabolic rate has been measured using direct calorimeters [102–104] and closed-circuit indirect calorimetry systems [105], but it is now generally measured with an open-flow indirect calorimetry system (Fig. 4.5). Room air is drawn through a clear plastic hood covering the individual's face, and the flow and concentration of oxygen and carbon dioxide in the intake and expired air are accurately measured for calculation of RMR [106]. This technique is known as indirect calorimetry because it does not directly measure heat but, rather, measures O_2 consumption and CO_2 production, which are then used to calculate energy expenditure. The pretesting environment impacts the measurement of RMR. Food, ethanol, caffeine, nicotine, physical activity, and room temperature impact RMR and should be controlled before measurements are taken [107]. A number of commercial indirect calorimetry systems are available, with some being more reliable than others [108]. Smaller, portable indirect calorimeters are also available, allowing investigators more mobility for field measurement of metabolic rate [109–111].

4 Estimating REE: Prediction Equations

Although numerous equations have been developed to estimate REE, the two most widely used equations are the Harris–Benedict equations [7] and those developed by the Institute of Medicine/Food and Nutrition Board to determine dietary reference intakes (DRIs).

a Harris–Benedict Equations

The Harris–Benedict equations remain the most commonly used tool by clinicians when estimating an individual's REE. The equations are often used as a basis for prescribing energy intake for hospitalized patients and to formulate energy intake goals for weight loss. A review of the data used in the formulation of the Harris–Benedict equations in the early 1930s deduced that the methods and conclusions of Harris and Benedict appear valid but not error-free [112]. The equations in use today are given in Table 4.1. Investigators have evaluated which equation performs best or developed new equations for specific populations [113–117]. The reader is encouraged to follow this literature as it applies to populations differing in body size, health status, race, sex, and age.

TABLE 4.1 Examples of Equations for Predicting Resting and BEE[a]

Harris–Benedict Equations	
Women	REE (kcal/day) = 655 + 9.56 (weight) + 1.85 (height) −4.68 (age)
	$r^2 = 0.53$, $F = 37.8$, $p < 0.001$
Men	REE (kcal/day) = 66.5 + 13.75 (weight) + 5.0 (height) −6.76 (age)
	$r^2 = 0.75$, $F = 135.2$, $p < 0.001$
DRI Equations	
Women	BEE (kcal/day) = 247 − (2.67 × age) + 401.5 × height + 8.6 × weight
	Residual = ± 156, $R^2 = 0.62$
Men	BEE (kcal/day) = 293 − (3.8 × age) + 456.4 × height + 10.2 × weight
	Residual = ± 156, $R^2 = 0.64$

[a]*Weight is in kilograms, height is in centimeters, and age is in years.*
DRI Equations for BEE: Institute of Medicine of the National Academies, Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, The National Academy Press, Washington, DC, 2005.
D.C. Frankenfield, E.R. Muth, W.A. Rowe, The Harris–Benedict studies of human basal metabolism: history and limitations. *J. Am. Diet. Assoc.* 98 (1998) 439–445.

b DRI Equations

The BEE prediction equations developed for the DRIs for lean, overweight, and obese men and women are given in Table 4.1. These equations were derived from observed BEE values found in the doubly labeled water (DLW) database [118]. This database was developed by searching published research that involved use of the DLW method. The investigators associated with the identified publications were solicited to contribute to the database. Twenty investigators responded and submitted individual TEE data and ancillary data including age, gender, height, weight, BEE (both observed and estimated), and descriptors for each individual in the data set. These data were not obtained from randomly selected individuals and thus are not a representative sample of the US population. However, because the measurements were obtained from men, women, and children, over a wide range of weights, heights, and ages, it is believed that the data still offer the best currently available information [118].

c Predicting REE in Disease and Physiological Conditions

REE has been characterized for a variety of disease states and physiological conditions, including burns [119–121], anorexia nervosa [122,123], severe central nervous system impairment [124], cerebral palsy [125], pregnancy [126], and lactation [127]. In addition, REE has been studied in both children [114] and the elderly [128–130]. Clinicians should not assume that prediction equations, which were developed in normal, healthy people, are valid in special populations [131].

d Thermic Effect of Food

TEF, sometimes referred to as diet-induced thermogenesis, represents a small (~10%) portion of TDEE (Fig. 4.1). TEF is the increase in energy expenditure that occurs after consumption of a meal. There are several protocols for measurement of TEF, but the general procedure utilizes a metabolic cart to measure metabolic rate for 3–6 hours after administration of a meal of ~35% of RMR, which is then compared to RMR measured either on the same day as the TEF or on a different day [64,100,132–135]. TEF is generally not measured in clinical settings but, rather, estimated as 10% of TEE. A major factor affecting the magnitude of the TEF is the macronutrient composition of the meal. When comparing isoenergetic protein, carbohydrate, or fat meals in lean and obese individuals, the TEF has been shown to be greater and more prolonged after a protein meal [136]. The TEF has also been shown to be higher after consuming mixed meals high in protein [137,138]. In a short-term (4 day) controlled metabolic chamber study of energy and substrate metabolism during consumption of a high protein diet (2.6 g/kg body weight) to maintain weight showed that sleeping metabolic rate, TEF, protein balance, and fat oxidation were increased, compared to an adequate (1 g/kg body weight) protein diet [139]. There is evidence that TEF is lower in children than in adults [64,100,140,141].

Whether TEF is lower in obesity is inconclusive. In one review of published TEF data, the authors concluded that TEF was lower in obesity, when appropriately accounting for known variables affecting TEF results, and that the lower TEF was associated with the degree of insulin resistance [142]. In a subsequent review of available literature by different investigators, the authors concluded that discrepant results and inconsistencies in methodology preclude a proper evaluation of the theory that TEF is lower in obesity [134].

e Energy Expended in Physical Activity

EEPA is the most variable component of total energy expenditure, varying between 100 and 800 kcal/day even in the confines of a metabolic chamber [143]. EEPA includes energy expended in voluntary exercise, which includes activities of daily living (e.g., bathing, feeding, and grooming), sports and leisure, and occupational activities. EEPA also includes the energy expended in nonexercise activity thermogenesis, which is associated with fidgeting, maintenance

of posture, and other physical activities of daily life [144]. Because of the alarmingly high rates of obesity in the United States, increasing EEPA through voluntary physical activity is being stressed as a potential method of achieving or maintaining a healthy weight [145].

5 Determinants of EEPA

Differences in EEPA are due both to patterns of activity and to body size and composition. The energy cost of activities in obese individuals is higher than in lean individuals due to higher body weight [146–148]. The energy expenditure (above resting) of several activities has been shown to be proportional to body weight [149]. For example, the gross energy cost of walking at the preferred walking speed has been shown to be similar in lean and obese men and women on a per kg basis (between 2.81 and 3.04 J/kg/m) [148]. The preferred walking speed was similar in obese and normal-weight individuals (1.41 m/s vs 1.47 m/s). Therefore, comparing the energy cost of walking for 1 hour at 1.4 m/s in a typical lean (63 kg) and Class III (124 kg) individual, using a gross energy cost of 3 J/kg/m, reveals that the energy cost of walking is nearly twice as high in the Class III obese individual (448 kcal vs 228 kcal). However, the EEPA recently observed in Class III obese individuals was only 20–25% higher than in lean individuals (Fig. 4.2) [20]. This is much lower than would be expected in light of the increased body weight ($\sim$100% higher in Class III compared to lean) if the obese were as active as the lean, suggesting that the obese individuals spent less time engaged in physical activity. Confirming that the obese individuals were less active, the total time spent in moderate-to-vigorous physical activity (1.2 ± 0.9 vs 2.5 ± 1.2 hours/day), and average daily metabolic equivalent (MET) level (1.1 ± 0.2 vs 1.6 ± 0.3 MET) were lower in the Class III obese compared to lean individuals [20].

When comparing the volume (intensity $\times$ time) of physical activity between individuals, a physical activity index has been calculated by dividing EEPA by body weight [149,150]. This approach has been utilized to compare EEPA in individuals with Prader–Willi syndrome [151] and when comparing EEPA in a wide variety of individuals across multiple studies [152].

Physical activity can also affect RMR in the postexercise period by 5% or more, up to 24 hours after exercise [153]. A decrease in the level of physical activity has been observed during the transition from childhood to adolescence [64,154]. A decrease in EEPA has also been observed in aging [130,155]. Cross-sectional and longitudinal studies have shown increases in body fat and decreases in muscle mass in older adults, often in the absence of differences or changes in body weight [156]. These changes in body composition are often associated with changes in physical function and metabolic risk. Fortunately, moderate-intensity PA intervention has been shown to improve physical function in older adults, and free-living activity energy expenditure has been shown to be strongly associated with lower risk of mortality in healthy older adults [157,158]. Table 4.2 provides average energy costs of typical activities, with the values expressed as multiples of RMR.

TABLE 4.2 Examples of Activity Energy Costs

Activity	Energy Cost (Multiple of Basal Metabolism)
Lying quietly	1.0
Riding in a vehicle	1.0
Light activity while sitting	1.5
Walking (2 mph)	2.5
Watering plants	2.5
Walking the dog	3.0
Cycling leisurely and household tasks (moderate effort)	3.5
Raking the lawn	4.0
Golfing (no cart) and gardening (no lifting)	4.4
Walking (4 mph)	4.5
Dancing, ballroom (fast), or square	5.5
Dancing, aerobic, or ballet	6.0
Walking (5 mph)	8.0
Jogging (10-min miles)	10.2
Skipping rope	12.0

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6 Measuring EEPA

Obtaining a valid and appropriate measurement of EEPA is a challenging task. Measures are classified into three general categories: objective assessment tools, including the DLW technique combined with RMR, activity monitors, and heart rate monitoring; direct observation; and subjective reports, such as physical activity questionnaires. The objective activity assessment tools are often used to validate the subjective activity measures.

a Objective Measures of EEPA

Doubly Labeled Water EEPA can be assessed by measuring TEE by DLW and subtracting measured RMR and either measured or estimated (10% of TEE) TEF [20,64,87]. DLW has been used as a gold standard for validating other methods to measure EEPA in free-living individuals. DLW measurement of TEE is discussed in detail in Section III.

Activity Monitors A variety of devices (e.g., accelerometers and heart rate monitors) can provide minute-by-minute information regarding physical activity patterns. However, these devices have limitations that affect their validity for assessing EEPA [159–163]. Accelerometers are movement counters that can measure body movement and often intensity. Accelerometers cannot be used to measure the static component in exercises such as weight lifting or carrying loads. However, in normal daily life, it is assumed that the effect of static exercise on the total level of physical activity is negligible [164]. Since 1999, there has been a push to improve accelerometers because it was concluded that those available were not practical for large-scale studies due to high cost, uncertain reliability, and difficulties in interpreting data [165]. NHANES 2003–04 began using accelerometers on survey participants ages 6 years or older who agreed to wear them, representing the largest implementation of objective physical activity monitoring [165].

Whereas accelerometry has been shown to be accurate for estimating energy expenditure during level walking, it consistently underestimates energy expenditure during other forms of activity [159,166,167]. In addition, although many devices/algorithms have been shown to perform well in a laboratory setting, they perform less well under free-living conditions. New regression models have been proposed to improve estimated energy expenditure using accelerometry [168,169]. In addition, new, multisensor devices have been developed that show promise, but they require further refinement and validation [170–175]. Newer consumer versions of activity monitors are now available and have been compared against with research-grade devices, indirect calorimetry, and DLW [176–178].

Heart Rate Monitoring The use of heart rate monitoring as a proxy measure for EEPA is based on the principle that heart rate and oxygen consumption tend to be linearly related throughout a large portion of the aerobic work range. Heart rate monitors are often used to assess EEPA because the technique is relatively inexpensive and easy to use. One drawback is that the relationship between heart rate and oxygen consumption must be characterized for each subject for several exercise intensities [179]. Even when conducting individual calibration relationships and using complex modeling, heart rate monitoring can provide inaccurate estimates of energy expenditure compared to DLW and indirect calorimetry [161,180,181]. Investigators have combined heart rate monitoring and accelerometry to provide more accurate measures of EEPA [174,175].

Metabolic Chambers Metabolic chambers generally have radar sensors for detecting physical activity. The measured EEPA assessed in a metabolic chamber has been shown to be quite variable, varying between 100 and 800 kcal/day [143]. The physical activity assessed in a metabolic chamber is considerably lower than free-living physical activity, and is limited to activities of daily living due to the small confines of the chamber. However, the EEPA measured in a metabolic chamber has been shown to be correlated with EEPA measured under free-living conditions [182]. Fifty nondiabetic Pima Indians were studied during a 24-hour stay in a metabolic chamber followed by 7 days of free-living conditions during which TEE was assessed by DLW and EEPA calculated by subtracting TEF and sleeping metabolic rate from TEE. As expected, EEPA in the chamber was significantly lower (440 ± 160 kcal/day) compared to EEPA assessed under free-living conditions (930 ± 310 kcal/day). However, there was a significant correlation between the two measures ($r = 0.53$, $p < 0.0.01$) in men and women.

b Physical Activity Questionnaires

Physical activity questionnaires have been used in many studies because they are easy to administer to large numbers of people and do not intrude on people's everyday activities. Although questionnaires do not provide precise estimates of EEPA, they may be helpful in ranking groups of subjects from the least to the most active. The ranking can then be used to correlate activity levels with disease outcomes [144]. An accurate questionnaire is both reliable and valid. A reliable questionnaire consistently provides similar results in the same circumstances, whereas a valid questionnaire truly measures what it was designed to measure. The validity of a physical activity questionnaire should be determined by comparing it with an objective measure of EEPA such as the DLW method. Starling et al. [183] found that the Yale Physical Activity Survey estimates of EEPA compared favorably with DLW on a group basis. However, its use as a proxy measure for individual EEPA is limited. In the same study, the Minnesota Leisure Time Physical Activity Questionnaire significantly underestimated EEPA in free-living older men and women [183]. This highlights the importance of ascertaining the validity of a questionnaire before applying it in large epidemiological studies.

III TOTAL ENERGY EXPENDITURE

Measured TEE provides an estimate of energy requirements when individuals are in energy balance. If significant weight gain or loss occurs during a metabolic study, changes in body energy stores must be accounted for to assess energy intake [3].

A Measuring Total Energy Expenditure

1 Indirect Calorimetry

Metabolic chambers, also known as respiratory chambers, are utilized to study energy expenditure over periods of time from 24 hours to several days. The concepts of metabolic chambers are similar to that of metabolic carts, requiring measurement of CO_2 and O_2 concentrations and the flow rate through the chamber to calculate oxygen consumption, carbon dioxide production, respiratory quotient (RQ), and metabolic rate [2,143,184–186]. Metabolic chambers provide accurate measures of 24-hour and sleeping energy expenditure, as well as long-term substrate utilization. However, due to the confined environment of the chamber, they do not provide an estimate of free-living energy expenditure.

2 Doubly Labeled Water

The introduction of the DLW technique for use in humans in 1982 by Schoeller and van Santen [187] provided a scientific breakthrough in the measurement of TEE in free-living humans. The method was originally described by Lifson et al. in the 1950s for use in rodents [188,189]. The method is based on the principle that a dose of $^2H_2{}^{18}O$ mixes with total body water, and the oxygen atoms in body water are also in equilibrium with exhaled CO_2 [190]. The isotopes undergo differential elimination from the body, with ^{18}O eliminated as carbon dioxide and water and deuterium eliminated only as water. The difference between the elimination rates of oxygen and hydrogen from body water are used to calculate CO_2 flux and, hence, energy expenditure. The DLW method has been extensively validated by a number of investigators throughout the world and shown to be accurate and precise [191]. For example, very good agreement was observed between 24-hour energy expenditure using DLW (1934 ± 377 kcal/day) and metabolic chambers (1906 ± 327 kcal/day) during caloric restriction [2].

a DLW Details

The application of the DLW method can be quite flexible depending on the individuals studied, the questions being asked, and balancing of the ideal protocol with costs and subject burden. Baseline urine samples are collected followed by oral administration of the $^2H_2{}^{18}O$ dose. Initial urine samples are obtained 4–6 hours following dose administration and again at the end of the study period for measurement of final isotopic enrichment. The length of a DLW study depends on the turnover of the two isotopes, which is driven primarily by water turnover. The optimal period for studies in normal adults is 7–14 days. If measurement of energy expenditure over a longer period of time is necessary, individuals can be re-dosed with DLW [192].

The ^{18}O and deuterium isotope abundances are measured after appropriate sample preparation using a gas-inlet isotope ratio mass spectrometer (IRMS) [192]. Advancements in automated devices to prepare and introduce samples into the mass spectrometer have greatly improved the precision and throughput of the isotope analyses. For example, Fig. 4.6 shows an H-Device system for injection of purified urine sample into a chromium reactor heated to 850°C for conversion to hydrogen gas before being introduced into the IRMS (just below the H-Device) for measurement of deuterium enrichment. A GasBench system (Fig. 4.6, right) is used to equilibrate a cleaned urine sample with carbon dioxide gas before being introduced into a gas chromatograph to purify the CO_2 and then introduced into the IRMS for measurement of ^{18}O enrichment. The CO_2 production rate is estimated using the measured isotope dilution spaces and elimination rates [191,193]. Energy expenditure is calculated utilizing the energy equivalent of CO_2 for a typical RQ such as 0.86 or using a calculated RQ from the macronutrient content of the diet consumed and body energy stores used during the DLW measurement period [192].

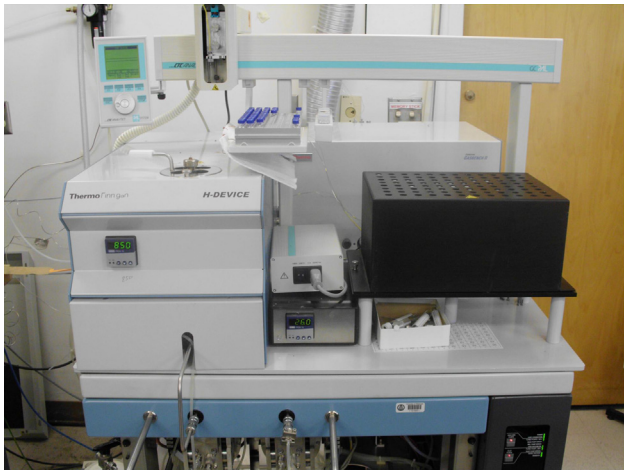

FIGURE 4.6 Mass spectrometry system used for the analysis of isotope enrichments for the DLW method.

TABLE 4.3 Advantages and Disadvantages of the DLW Technique to Measure Total Energy Expenditure

Advantages	Disadvantages
Noninvasive, unobtrusive, and easily administered	Availability and expense of oxygen-18 (varies between $300 and $1000 for 70-kg adult)
No reliance on participant to do anything except drink the labeled water and provide timed urine samples	Need for isotope ratio mass spectrometry for analysis of samples
Measurement performed under free-living conditions over extended time period (7–14 days)	A direct measure of CO_2 production, so need an estimate of RQ
Accurate and precise (2–8%)	Expensive for large-scale epidemiological studies
Can be used to estimate EEPA when combined with measurement of RMR	Does not provide information regarding time spent in activity or intensity of activities
Longitudinal measures of DLW and body composition can be used to objectively assess energy intake	

TABLE 4.4 Doubly Labeled Water Data for Individuals With a Body Mass Index (BMI) in the Range From 18.5 to 25 kg/m^2

Age Group (years)	*n*	Mean BMI (kg/m^2)	TEE (kcal/day)	BEE Mean (kcal/day)	Mean Physical Activity Level (TEE/BEE)
Females					
3–8	227	15.6	1487	1035	1.57
9–13	89	17.4	1907	1320	1.68
14–18	42	20.4	2302	1729	1.73
19–30	82	21.4	2436	1769	1.70
31–50	61	21.6	2404	1675	1.68
51–70	71	22.2	2066	1524	1.69
71 +	24	21.8	1564	1480	1.62
Males					
3–8	129	15.4	1441	1004	1.64
9–13	28	17.2	2079	1186	1.74
14–18	10	20.4	3116	1361	1.75
19–30	48	22.0	3081	1361	1.85
31–50	59	22.6	3021	1322	1.77
51–70	24	23.0	2469	1226	1.64
70 +	38	22.8	2238	1183	1.61

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b Advantages and Disadvantages of the DLW Technique

Advantages of the DLW method (Table 4.3) are that it is a true field technique for accurate and precise (2–8%) assessment of free-living energy expenditure that requires no subject compliance, can be used to validate other techniques, and when combined with other methods can provide measures of EEPA, as described earlier. The DLW method also provides an objective criterion method for validation of more subjective estimates of energy expenditure and energy intake.

Disadvantages of the DLW method include the cost and availability of the ^{18}O-labeled water, need for expensive IRMSs and sample preparation systems, and technical difficulties with accurate measurements of isotope enrichments [194]. Another disadvantage of the DLW method is that it provides no information regarding time spent in physical activity, activity patterns, or intensity. Therefore, when conducting a DLW study, it is extremely advantageous to include other methodologies, including RMR so that EEPA can be assessed, as well as activity monitors so that activity intensity and patterns can also be assessed.

c Estimating Total Energy Expenditure

The introduction of the DLW technique in humans has produced a large and robust database of TEE measurements in a variety of populations. A meta-analysis of 574 DLW measurements helped to establish the average and range of habitual energy expenditures in different age and sex groups [155]. Since this study, many DLW studies have been completed. The Food and Nutrition Board used data from these DLW studies to compile a worldwide TEE database [118]. Table 4.4 is a summary of data from this database. The database provides a frame of reference for energy needs in the general population and can be used to evaluate other estimates of energy expenditure. Special circumstances such as illness or enforced exertion were excluded from this database.

Because of the high cost and small numbers of laboratories with the capacity to do DLW studies, clinicians continue to rely heavily on prediction equations to estimate energy requirements. The TEE database compiled for the DRIs was used to derive equations for predicting

TABLE 4.5 Equations to Predict Energy Requirements in Females and Males 19 Years and Older

For Females
EER = 354.1 − 6.91 × age + PA × (9.36 × weight + 726 × height), where PA is the physical activity coefficient:[a]
PA = 1.00 if PAL is estimated to be ≥1.0 < 1.4 (sedentary)
PA = 1.12 if PAL is estimated to be ≥1.4 < 1.6 (low active)
PA = 1.27 if PAL is estimated to be ≥1.6 < 1.9 (active)
PA = 1.45 if PAL is estimated to be ≥1.9 < 2.5 (very active)
For Males
EER = 661.8 − 9.53 × age + PA × (15.91 × weight + 539.6 × height), where PA is the physical activity coefficient:
PA = 1.00 if PAL is estimated to be ≥1.0 < 1.4 (sedentary)
PA = 1.12 if PAL is estimated to be ≥1.4 < 1.6 (low active)
PA = 1.27 if PAL is estimated to be ≥1.6 < 1.9 (active)
PA = 1.45 if PAL is estimated to be ≥1.9 < 2.5 (very active)

[a]*Weight is in kilograms, height is in centimeters, and age is in years.*
Institute of Medicine, Dietary Reference Intake for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, National Academies Press, Washington, DC, 2005.

energy expenditure requirements. These equations are provided in Table 4.5.

d Total Energy Expenditure in Special Populations

During approximately the past 20 years, there has been a proliferation of studies using DLW to examine TEE in various disease states, physiological conditions, and across the life cycle. Hence, data are now available on energy expenditure during infancy [195], childhood [64,196], adolescence [197], and in the elderly [130]. TEE during pregnancy and lactation has been well characterized in some elegant longitudinal studies [198]. In addition, TEE has been examined in adults and children with obesity [94,199,200]; infants and children with cystic fibrosis [201,202]; children treated for acute lymphoblastic leukemia (ALL) [203] with burns [204] and Down syndrome [205], and adults with cerebral palsy [206], neuromuscular disease [207] Alzheimer's disease [208], and HIV/AIDs [209]. The effects of these various conditions on energy requirements are highlighted in Table 4.6.

TABLE 4.6 Effects of Disease States and Physiological Conditions on Energy Requirements: Results from DLW Studies

Disease or Condition	Effect on Energy Requirements	Explanation
Aging	Decreased	Decreased RMR, decreased EEPA in nonagenarians
Alzheimer's disease	No change	Energy expenditure not elevated, low-energy intake predisposes to weight loss
Anorexia	Increased	To counteract increased physical activity with underweight and hypometabolism
Burns	No change	Increased RMR counteracted by decreased physical activity
Cerebral palsy	Relative to individual	High interindividual variation in EEPA; ambulation status an important predictor
Children treated for ALL	Decreased	Reduced physical activity
Cystic fibrosis	Increased	Mechanism unknown
HIV/AIDS	No change	Energy expenditure not elevated, reduced energy intake causes weight loss
Lactation	Increased	Energy cost of milk production partially offset by reduced physical activity
Neuromuscular disease	Decreased	No difference in RMR, but TEE decreased
Obesity	Increased	Increased FFM and fat mass, but decreased time spent in physical activity
Pregnancy	Relative to individual	No prediction of metabolic response
Spinal cord	Decreased	Lower EEPA, RMR, and TEF

e Use of DLW to Estimate Energy Intake

Although knowledge of energy intake is essential for understanding energy balance, true dietary intake is consistently underreported, leading some to question whether investigators should discontinue the use of self-reported intake methods [210,211]. For example, the Observing Protein and Energy Nutrition Study in

484 men and women compared a food frequency questionnaire (FFQ) and a 24-hour recall (24HR) with DLW [1]. On average, men underreported energy intake by 12−14% using 24HR and 31−36% using FFQ. Women underreported energy intake by 16−20% using 24HR and by 34−38% using FFQ. Similar levels of underreporting (17−33%) have been observed in children when comparing 8-day food records with DLW, and greater underreporting has been observed in obese compared to lean children [212]. Even those defending self-reported intake, pointing out important information that has been gained using these instruments acknowledge that self-reported energy intake should not be used as a measure of true energy intake [213].

Objective measures of energy intake can be obtained by the intake-balance method, by measuring energy expenditure and changes in energy body stores. While estimation of TEE by DLW during over a 2-week period is accurate and precise, measurement of changes in body energy stores is considerably less accurate due to the relative error in measurements of fat mass and FFM [2]. However, if longer-term studies are conducted, with TEE and body composition measures conducted at baseline and 6 months later, the errors associated with body composition measures are negligible. This technique has been used during a multicenter 6-month study of caloric restriction [3], in a study to examine the effects of physical activity on energy expenditure and energy intake during behavioral weight loss intervention [87], in a study to explore reasons for the lower weight loss observed in African-American women during intervention [214] and during overfeeding [215].

IV RECOMMENDED ENERGY INTAKES

Classically, the Recommended Dietary Allowances (RDAs) have been used as a guide to determine energy intakes for groups of normal, healthy people. The last RDA for energy, which was labeled the recommended energy intake (REI), was set in 1989. RDAs for all nutrients except energy are set at levels well above those estimated to minimize the occurrence of deficiency syndromes. For energy, this obviously is not the case because there are adverse effects to individuals who consume energy above their requirements over time resulting in weight gain. Recommendations for energy have always been set as an average of energy requirements for a population group. In the past, REIs relied heavily on data from dietary surveys that estimate energy intake. This is based on the assumption that people are in energy balance at the time of measurement and that the estimates of energy intake are valid. A large body of evidence now demonstrates that self-reported estimates of food intake do not provide accurate or unbiased estimates of people's energy intake and that underreporting of food intake is pervasive in children, teens, and adults [212,216,217].

The DRIs [118] adopted an alternative approach and instead summarized data from multiple DLW studies to estimate energy requirements. There is no RDA for energy because it would be inappropriate to recommend levels that would exceed requirements of 97% or 98% of individuals. Instead, the requirement for energy for individuals of normal weight is expressed as an estimated energy requirement (EER), which reflects the energy expenditure based on the individual's sex, age, height, weight, and physical activity (see Table 4.5 for equations). For overweight individuals, these equations estimate TEE rather than the EER, which is reserved for normal-weight individuals. The equations are estimates of energy needs based on maintaining current weight and activity level and therefore were not designed to lead to weight loss in overweight individuals [118].

As the prevalence of obesity has reached epidemic proportions in the United States, people's EEPA has become so low that it may become increasingly difficult to meet the micronutrient needs on the energy intakes required to keep people in energy balance. Hence, it will become increasingly imperative that emphasis be placed on increased EEPA for the maintenance of optimal health.

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Chapter 5

Metabolomics

G.A. Nagana Gowda[1], Leiddy Z. Alvarado[2] and Daniel Raftery[1,3]

[1]*University of Washington, Seattle, WA, United States,* [2]*Albany Molecular Research Inc., Rensselaer, NY, United States,* [3]*Fred Hutchinson Cancer Research Center, Seattle, WA, United States*

I INTRODUCTION

The field of metabolomics focuses on the study of the multiple of small molecules present in biological samples such as biofluids, cells, and tissues, as well as plants, foods, etc. It offers new avenues for understanding biological phenotypes, deciphering mechanisms, and identifying biomarkers or drug targets for a variety of conditions [1,2] (Fig. 5.1). Metabolites represent the downstream products of gene expression and protein action, and thus provide an instantaneous snapshot of biological phenotype. Changes in metabolite levels as well as their transformation rates (fluxes) in biological systems result from a variety of stresses including disease (pathophysiological stimuli) [3,4]. Metabolomics also offers a window into the downstream biochemical changes that result from a combination of environmental exposures and genetics [3,4]. In addition to studies and developments in related "omics" fields such as genomics and proteomics, further information on the molecular products' biological stresses or perturbations is highly desired for disease detection, diagnosis, prognosis, and development of preventive screening methods. Over the last several decades, the field of metabolomics has developed as a result of significant technological advances for measuring and interpreting metabolic changes. As a result, applications in a growing number of areas have been reported, including, but not limited to, toxicology, pharmacology, early disease diagnosis, drug target identification and development, environmental studies, as well as the study of food and nutrition [2,3,5]. The perturbed metabolites can be localized to specific pathways that may be related to protein or gene (dis)function as well as to various disease states. Thus, metabolomics can reveal significant information that is closely related to existent diseases or health disorders, or even to therapeutic status. All of these aspects and opportunities have made the metabolomics field a vibrant area of research.

The wide range of applications resulting from metabolomics-based research arises from the ability to detect up to many hundreds of metabolites and their concentration and flux changes in a variety of biological specimens. More than 30,000 endogenous human body metabolites have been identified thus far, with a majority belonging to various lipid classes [6]. Plants are known to have secondary metabolites that increase this number to over 200,000; however, many of these exist at very low concentrations. Therefore, advanced analytical methods are required to profile a broad range of metabolites that constitute the "metabolome." By using highly sensitive mass spectrometry (MS) techniques, up to a few thousand metabolites can be routinely detected by global analysis; however, less than one-third of these are identified in practice with a vast majority of the signals remaining unidentified. Other methods, such as nuclear magnetic resonance (NMR), or more targeted MS approaches detect a small fraction of the metabolome as discussed below, leaving reliable detection and identification of the complete metabolome as one of the major challenges in the field of analytical chemistry. Nevertheless, significant technological advances in the last decade have pushed metabolomics methods and applications to new heights.

A Brief History

Although metabolomics is considered a relatively new field, as early as 500 BC Hippocrates was one of the first physicians to interpret the functions of the human body based on urine characteristics; further, some accounts indicate that urine-based diagnosis even predates Hippocrates [7]. As early as 4000 BC, one of the earliest

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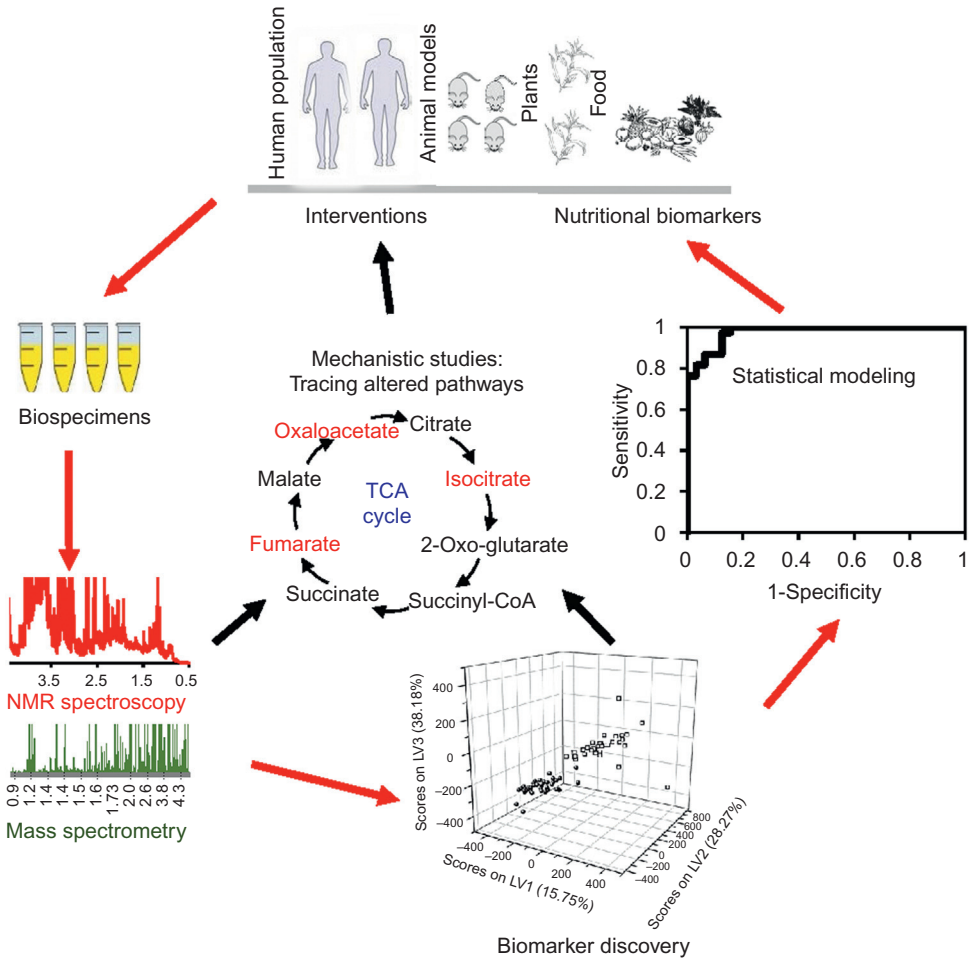

FIGURE 5.1 Schematic diagram depicting the application of NMR and MS-based metabolic profiling for early disease diagnosis, systems biology research, and discovery of dietary and nutritional biomarkers and drug targets.

civilizations is believed to have recognized altered characteristics of urine in different diseases [8]. It is also known in Hindu culture that some people's urine tasted sweet, and that black ants were attracted to this sweet urine, which is now known to be a symptom of the widespread disease "diabetes" [9]. In ancient China, it was known that ants could be used to detect diabetes based on urine samples [10]. In the Middle Ages, "urine charts" that correlated smell, taste, and color of urine were employed to diagnose various medical conditions now known to be of metabolic in origin [11]. Distinctive "metabolic patterns" that could be "finger-printed" by studying biological fluids was proposed in late 1940s [12]. In these investigations, paper chromatography was utilized to demonstrate significant variations of metabolic patterns among different subjects including alcoholics and schizophrenics.

The growth of metabolomics technologies benefited by the detection of inborn errors of metabolism (IEM). Specifically, from 1950s to 1970s the field witnessed explosive growth in the number of IEMs detected [13]. A factor that contributed to modern metabolomics is the metabolic-control analysis developed in the 1960s for modeling metabolism in cells, which used metabolite concentrations derived often using gas chromatography (GC) or GC coupled to MS [11]. Another contributing factor was the development of NMR spectroscopy methods, which in the 1980s were able to identify metabolites in intact biological fluids. The technological advances in GC, liquid chromatography (LC), and MS in the 1960s and 1970s enabled quantitative metabolic profiling studies. In 1971, Horning and coworkers used gas chromatography–mass spectrometry (GC-MS) to measure metabolites in human urine and tissue extracts [14,15]. Horning, along with Pauling and Robinson, led the development of GC-MS-based techniques focused on the measurements of metabolites in biological mixtures through the 1970s to early 1980s [16]. Subsequent developments

of high-resolution and high-sensitivity MS and NMR instrumentation in combination with multivariate statistical analysis set the stage for metabolomics to become one of the fast-growing fields in systems biology.

B Relevance to Nutrition

Metabolomics is widely used to investigate numerous aspects of nutrition using a variety of samples ranging from plants and foods to human and animal tissues and biofluids. Plant metabolomics is increasingly realized as an important tool for investigations of systems biology [17]. Metabolic profiling of plants has been applied to numerous key areas, including growth, development, and stress responses, the discovery of phenotypic differences induced by genetic variations or environmental perturbations, in environmental studies for monitoring the response of wild species to changes such as temperature, human activities, diseases, and pollutants, as well as many other areas [17–20].

The application of metabolomics in food science has also seen significant advances. Owing to its direct relation to nutrition and human health, food metabolomics is witnessing increased interest in the recent years. To date, a variety of foods including fruits, vegetables, beer, and meats have been subjected to metabolomics investigations [21]. One area of food metabolomics evaluates food quality, since metabolite fingerprints provide information on the composition of foods and allow characterization and authentication of foods [22,23]. Numerous studies have focused on understanding the importance of food for health for reducing (or increasing) the risk of diseases. Diet is known to significantly impact human health and hence there is an important need for comprehensive understanding of metabolites present in foods. Such understanding will positively influence food production by enabling efforts to favorably modulate the food metabolome. Thus, numerous metabolomics studies have focused on understanding the impact of agricultural practices, processing, and storage on the metabolite composition of food; studies have also focused on identification of novel bioactive compounds and determination of their regions of origin [21].

II SPECIMENS

A variety of biological samples are investigated by metabolomics due to the significant amount of information that can be extracted, from each type of sample, on metabolic changes and their connections with biological perturbations. Blood (serum or plasma) and urine top the list owing to their high information content combined with the ability to obtain these biofluids with noninvasive or minimally invasive methods. Analyses of tissue and cell samples have also gained strong interest for understanding cellular function and for the identification of potential drug targets. Moreover, fecal samples are being used to investigate the gut microbial community. The analysis of plant and food samples forms a major basis for the development of nutritional metabolomics. Environmental samples as well as many other sample types are also among those widely investigated.

A Blood Serum and Plasma Samples

Among the biological fluids commonly studied in metabolomics, blood serum and plasma are most often used due to the fact that they are rich in metabolites that are capable of providing information on disease, toxicity, and pathophysiological changes. Due to the compositional complexity of blood, which is sensitive to many factors relevant to health and diseases, extensive efforts in sample selection, collection, preparation, and metabolite extraction have been made [24]. As an easily accessible and important biological fluid, blood has been the subject of comprehensive studies over decades and is used for many clinical metabolic determinations [25].

Blood serum and plasma are metabolite-rich biological media but they also contain a large concentration of macromolecules such as proteins and lipids. Macromolecules pose challenges particularly for analysis using MS. Therefore, blood serum/plasma are generally deproteinized by the addition of organic solvents such as methanol or acetonitrile, as a necessary step during sample preparation [26,27]. Blood treatment conditions such as clotting time and temperature, centrifugation speed and time, the type of vials used during sample collection and preparation, and sample storage are important parameters that need to be carefully controlled in order to obtain reliable and reproducible results.

B Urine Samples

Urine contains a wide variety of metabolites, which are generated by a large range of cellular metabolism. It is particularly advantageous to study urine samples due to their ready accessibility owing to the noninvasive collection. While water-soluble metabolites are the primary components of urine, many other components such as inorganic ions and non-polar organic solutes including trace levels of enzymes and fatty acids are present. Variations in urine content can be seen between individuals depending on food intake, urine concentration, and other environmental and physiological factors, which can complicate data analysis. Therefore, a systematic sample collection, storage, and preparation protocol needs to be performed in order to obtain stable and reliable results, although careful normalization procedures (see below)

can counteract the deleterious effect of dilution. Due to the relatively low abundance of high molecular weight compounds in urine, minimal sample preparation is required for high quality measurements, especially for NMR analysis. For NMR experiments, peak position (i.e., chemical shift) variations due to different salt concentrations can be observed, while for MS, high salt concentrations can lead to adduct formation and signal suppression, and can negatively affect the performance of chromatography coupled-MS instruments. The above-mentioned effects can be minimized by sample pretreatment (including desalting) prior to data acquisition analysis [28].

C Cell Samples

Metabolomics studies of cell samples have revealed a number of new mechanistic insights and even drug targets in cancer and other diseases [29,30]. In the area of methods development, advances in sample preparation, treatment, and other protocols allow the discrimination of intracellular and extracellular metabolites and the measurements of metabolic fluxes using isotope tracers [31]. Careful treatment procedures must be followed when dealing with cell samples as several types of cells are known to be highly sensitive to osmotic changes of their surrounding medium, while the effects of different solvents can have a profound effect on metabolite recovery and even stability. NMR and matrix/assisted laser-desorption ionization time-of-flight MS (MALDI-TOF-MS) analyses have been performed on intact cell samples, while extraction methods usually using organic solvents are very popular for liquid chromatography−mass spectrometry (LC-MS), GC-MS, and also NMR analyses.

D Tissue Samples

A variety of tissue samples have been investigated using a broad range of analytical techniques in metabolomics, driven by the fact that the analysis of tissue metabolites potentially provides insights into the molecular basis of a variety of diseases, especially carcinogenic tumors [32]. High-resolution magic angle spinning NMR spectroscopy has often been employed in metabolomics analysis of intact tissue samples when minimal sample preparation is desired. Using this approach, several malignancy markers have been reported in a variety of cancer types [32,33]. Advancements in MS ionization methods such as DESI (desorption electrospray ionization) have made it possible to analyze tissue samples directly, including during surgery [34,35]. While analysis of tissue biopsies represents a direct examination to better understand altered metabolism, it is a less popular approach due to the invasive nature required to obtain the samples.

E Plant and Food Samples

Metabolomics is also applied to other complex biological mixtures such as plants and food samples. Broadly, the metabolomics analysis of plants involves collecting metabolite data of unpurified plant extracts and then analyzing the data using multivariate statistical methods, as will be described below briefly, as they have been provided previously in significant detail [36]. Plant extraction procedures must recognize the potential for degradation or modification of metabolites by enzymes released as well as other factors during extraction and sample preparation. Therefore, one should first rapidly cool the plant tissue to extremely low temperature to restrict enzyme activity and, subsequently, disrupt the tissue and inactivate enzymes by denaturation with acid or organic solvents [37]. A number of factors affect the reproducibility and it is important to consider each factor carefully to obtain reliable results. Important factors include, among others, the position of the leaf, its age, exposure to sunlight and rain as well as the time of collection, and the weather [36]. For analysis of food samples, metabolites extraction is required prior to analysis using NMR or MS method. Different solvent systems with various polarities, including methanol, acetonitrile, ether, acetone, hexane, or cyclohexane can be used, each of which provides complimentary information on the metabolome [38]. However, in case of liquid samples such as wine and beer, intact samples are generally used especially for analysis using NMR [39,40].

F General Study Design

Prior to the collection of samples it is very important that the study be well designed. For example, variations such as age, gender, race, weight, life style, medication use, and underlying diseases can alter the metabolic profiles, and as such, it is important to minimize unwanted sources of variation when designing metabolomics studies. Accordingly, the study design of metabolomics must be well-planned and carefully performed using sufficient numbers of reliably matched samples so that the investigation can use its full analytical capabilities. The inclusion of good quality control samples and standards/calibrants is also part of good analytical method protocols for reliable data acquisition [41,42]. A number of resources exist that describe the process for biomarker discovery in general, under the heading Quality Control [43,44].

A good study design should also incorporate proper validation protocols. One aspect of validation is to make

the samples blinded to the analyst; another is to use different analytical platforms to analyze the same samples. Further, most biomarker discovery efforts in the metabolomics field rely on relatively small numbers of samples owing to the cost and effort required to acquire such samples. Information derived from small sets of samples runs the risk of overriding confounding effects or false discoveries. Generally, validation involves dividing samples into two groups, training and validation sets. While a prediction model is developed using the training sample set, the validation set is used to evaluate model performance. Independent sample sets for validation help reduce confounding factors that arise in the analysis stage. Good study design should incorporate steps to account for both biological and technical variance, and provide important information on biomarker performance including sensitivity and specificity.

G Sample Preparation

Sample preparation—prior to data acquisition—is a critical step in all metabolic profiling studies. A typical procedure consists of biological sample collection (which may have its own detailed protocols including amounts, timing, collection tubes, etc.), storage, and typically, metabolite extraction prior to analyzing the samples. Given the complexity of biological samples, metabolite extraction is often a key step in performing metabolic profiling. Blood serum and plasma specimens contain a wide variety of proteins such as lipoproteins; on the other hand, urine contains high concentrations of urea and salts that can interfere with the low molecular weight compounds of interest. The ideal extraction procedure should remove interferences while being simple to carry out, and provide the maximum recovery of a broad range of metabolites (or analytes, more generally) as possible. Numerous extraction procedures have been utilized in the sample preparation of a variety of studies; some of these have been optimized to obtain the highest reproducible results possible [45], whereas others focus on the largest number of features or alternatively a more limited class of metabolites.

For MS analysis, generally, samples are extracted with an appropriate solvent, depending on the compounds of interest (such as just aqueous or lipid metabolites); otherwise, multiple extraction procedures can be applied for more global profiling approaches. For serum and plasma samples, organic solvents such as methanol or acetonitrile are added, which force the precipitation of proteins and many lipids. In contrast, chloroform or other nonpolar solvents can be used to extract lipid species. Urine samples are usually treated with urease to eliminate urea, and most importantly to avoid column overloading and potential ion suppression [46]. Using GC-MS, several biological fluids can be directly analyzed after extraction procedures; however, the low volatility of numerous metabolites make a chemical derivatization step important for broadening the metabolite coverage of the method [47].

Sample preparation for NMR-based analysis is somewhat simplified, and ranges from none to limited, depending on the type of biological specimen being analyzed. Several studies reported comprehensive procedures on sample collection, storage, and preparation for NMR-based metabolic profiling investigations [45,48]. NMR chemical shifts are well documented for common metabolites and are quite reliable. However, they can show some small dependencies on a number of factors such as pH, concentration, ionic strength, and temperature [49]. A phosphate buffer solution made up in D_2O is often used to reduce pH effects, while sodium azide (NaN_3) is commonly added to prevent bacterial growth in intact urine specimens.

III METABOLOMICS ANALYTICAL TOOLS

Among the analytical techniques that have been employed in metabolic profiling studies, MS and NMR are the most commonly utilized due to their ability to analyze a large number of metabolites in a single measurement with high throughput [50–52]. Moreover, the two methods possess unique characteristics; NMR is highly reproducible and quantitative, while MS is intrinsically highly sensitive, which makes it an important method for measuring metabolites present at even very low concentrations in complex biological samples. While NMR generally does not use chromatography, MS methods are most often combined with a separation technique such as LC, GC or capillary electrophoresis (CE) in numerous metabolomics investigations [50]. Some important characteristics of NMR and MS in relation to metabolomics studies are provided in Table 5.1.

A Mass Spectrometry

The use of MS-based techniques in metabolomics has continuously increased over the past decade, with an ever growing number of applications being investigated [28,53]. One of the major advantages of MS is that it offers quantitative analysis with high sensitivity and selectivity, which allows the use of relatively small amounts of sample [54,55]. The chromatographic separation techniques (LC, GC, or CE) normally associated with MS analysis reduce the complexity of the individual mass spectra. This effect, in turn, offers better opportunities for compound identification which can be achieved utilizing metabolite databases and libraries [56,57]. The high sensitivity (picogram level) of MS detectors makes MS a

TABLE 5.1 Characteristics of MS and NMR Spectroscopy Methods Used in Metabolomics Applications

	Strength	Weakness
MS	• Highly sensitive • Detects up to thousands of metabolites in a single measurement • Tandem mass analysis enables reliable metabolite identification • Highly quantitative using internal standards (one per metabolite) • Accurate mass can be used to derive empirical formulas • Enables high-throughput measurements • Wide range of instruments enables detection of different types of metabolites • Instrument cost can be relatively low	• Chromatographic separation is generally required before detection • Often, expensive internal standard is required for each metabolite for absolute quantitation • Salty samples are hard to analyze • Identities for a major fraction of detected metabolites are not known • Destructive to samples • High-end instruments are expensive
NMR	• Highly quantitative • Highly reproducible • Detects up to hundreds of metabolites in a single measurement • Generally no chromatographic separation is involved • Single internal reference is sufficient for absolute quantitation of all detected metabolites • Enables unknown metabolite identification de novo • Enables high-throughput measurements • Nondestructive to samples	• Relatively less sensitive (Limit of Detection $\sim 1\ \mu M$) • Limited spectral resolution • Detects fewer metabolites compared to MS • Difficulty measuring individual lipids • High cost for high field instruments

highly suitable analytical platform to investigate complex pathophysiological disorders in biological samples.

B Liquid Chromatography Resolved Mass Spectrometry

Due to its characteristics of high metabolome coverage and relatively simple sample preparation, LC-MS has become the most popular technology for metabolite profiling over the past five years. The intrinsic high sensitivity of MS enables detection of several hundred to over a thousand small molecules (along with thousands of unidentified spectral features) from a single experiment. The unique characteristics of LC-MS allow the direct detection of metabolites from biological mixtures with no requirement for chemical modification such as derivatization. Robust step-by-step protocols are available for LC-MS analysis of a variety of biological mixtures including serum [58], urine [59], as well as for animal and human tissue analysis [60]. More recently, detailed protocols for characterization of unknown peaks using tandem mass spectra along with a large database of over 50,000 metabolites (METLIN database) have been compiled [61].

A variety of LC columns are used to separate metabolites prior to mass analysis, depending on the nature of metabolites of interest. Reversed phase (RP) LC is primarily used for broad-based global metabolic profiling [62]. Although both polar and nonpolar metabolites can be separated by RPLC, highly polar metabolites are not well retained (separated) on common RP stationary phases. Instead, hydrophilic interaction chromatography MS is commonly used to analyze highly polar metabolites [63]. The development and use of ultrahigh pressure liquid chromatography has significantly improved the peak capacity and chromatographic resolution, and in so doing, lowered the limit of detection for metabolite profiling [64].

Postseparation by chromatography, metabolites are ionized in the MS ion source. The ionization source most commonly used for LC-MS-based metabolic profiling methods is electrospray ionization (ESI), which is operated in either positive or negative mode to ionize a broad range of metabolites. ESI is known as a "soft" ionization technique in that it typically does not result in a significant amount of molecular ion fragmentation. ESI alone does not allow for easy metabolite identification based on the MS fragmentation patterns. Therefore, ESI is very often coupled with detection with part per million mass resolution, to allow metabolite identification. Alternatively, tandem mass spectrometry (MS/MS), which includes an ion fragmentation step that produces additional molecular information, can assist in metabolite identification [65].

A variety of mass spectrometers are available for LC-MS analysis. High mass resolution instruments include Fourier transform ion cyclotron resonance, Orbitraps, and TOF instruments. Low (i.e., unit) mass resolution instruments include ion traps, Qtrap, and standard triple quadrupoles. High-resolution MS instruments provide important routes to detect different coeluting species and for unknown identification. A recent book describes different configurations and performance of these types of instruments along with their applications to metabolomics [50]. A significant challenge for LC-MS, however, is ion suppression in which coeluting compounds alter the degree of ionization of a particular analyte and cause serious signal attenuation or even signal loss.

C Gas Chromatography Resolved Mass Spectrometry

GC-MS is widely used in metabolomics due to numerous factors including its reliability and relatively low instrument cost and maintenance [57,66]. The high separation efficiency, resolution, and high reproducibility achieved through retention time markers represent important characteristics for GC-MS-based metabolite profiling. However, GC-MS is limited to the use of volatile and thermally stable compounds; those which are not volatile are often subjected to chemical derivatization to make them volatile and compatible with the GC interface [67]. As a result, GC-MS can be applied to the analysis of a large range of metabolite classes, including lipids, peptides, alcohols, organic acids, amino acids, ketones, aldehydes, esters, sulfides, sugars, sugar-phosphates, sugar-alcohols, alkaloids, amines, and amides. Due to the use of a gaseous mobile phase and the high energy nature of its ionization source (typically 70 eV), GC-MS analysis generally avoids the interfering effects of ion suppression on metabolite signal intensities, which is a major challenge faced by LC-MS. The detection of 150−200 identified metabolites along with a few hundred unknown metabolites and spectral features is typically achieved using GC-MS analysis of biofluid or tissue extract samples.

GC-MS analysis normally requires somewhat extensive sample preparation including chemical derivatization to increase thermal stability and volatility [68]. Metabolites with active hydrogens from the functional groups such as −COOH, −NH, −SH, and −OH can be derivatized by alkylation, acylation, or silylation. However, silylation is the most commonly used derivatization method, in which an active hydrogen atom is replaced by a silyl group. A number of silylation reagents are used, each of which has its own advantages and disadvantages. Depending on the experimental design and research interest, a two-step derivatization procedure is generally used, which includes methoximation to help derivatize keto- groups to prevent enolyzation, followed by silylation to cover a broad range of metabolites [69].

The ion source primarily used in GC-MS platforms is electron impact ionization (EI), which provides good, reliable, and reproducible metabolite fragmentation patterns for compound identification when used in conjunction with large and commercially available MS libraries. Some instruments are equipped instead or additionally with chemical ionization (CI). CI is generally considered to be a softer ionization source and usually gives the M^+ peak, instead of only molecular fragments commonly observed in EI. Most metabolomics-based investigations that use GC-MS instruments are equipped with either quadrupole or TOF mass analyzers [57]. TOF analyzers offer higher mass resolution and rapid metabolite detection, which is useful when dealing with narrow, unknown peaks in the chromatogram [70,71].

In order to improve and increase metabolite separation, the use of comprehensive, two-dimensional (2D) GC coupled to a mass spectrometer (GC × GC-MS) has also been developed and applied to metabolomics analysis [72]. Compared to GC-MS, the second chromatographic dimension in GC × GC-MS provides improved resolution and high sensitivity which is very advantageous for metabolomics applications. However, the data sets obtained from this platform are large (typically 100 MB per sample) and quite complex; improved solutions for peak deconvolution and data processing are only now becoming available [73].

D Other MS-Based Techniques

Additional MS-based techniques have been employed to analyze the metabolome, and numerous applications have been investigated. Among them, capillary electrophoresis-mass spectrometry (CE-MS) is a rapidly growing field in metabolomics that has already demonstrated great potential [74]. However, CE is limited to the analysis of charged molecules, and lower sensitivities are often obtained as compared with GC-MS or LC-MS. The application of MALDI, laser desorption ionization, and new atmospheric ionization methods, such as DESI [75], direct analysis in real time [76], and nano-ESI [77], have been investigated for application in metabolomics; these methods have potential for providing much simpler, less time consuming analytical approaches than LC-MS or GC-MS. However, these methods do suffer from a variety of challenges, such as reduced reproducibility, difficulty in standardization, and spectral overlap. Nevertheless, new applications of these approaches continue to be developed. For example, the utility of the DESI approach has recently been demonstrated in the surgical room. This approach, known as iKnife, can detect volatized metabolites from a special

surgical knife that can be analyzed to indicate the presence of cancer or healthy tissue essentially in real time [34]. Although a single analytical platform can be suitable for performing metabolite profiling studies, the use of multiple-platform approaches has gained much interest over the past decade, as a wider range of metabolites can be analyzed using this approach [52,78,79].

E NMR Spectroscopy

High-resolution NMR spectroscopy has proven to be a powerful and very reliable technique for metabolite profiling [51,52]. A growing number of metabolomics studies have demonstrated the capabilities of NMR in a variety of fields including toxicology, drug discovery, disease diagnostics, environmental studies, energy, and foods and nutrition. NMR spectroscopy exhibits numerous unique and favorable characteristics that are very beneficial to the field of metabolomics [52]. Importantly, (1) NMR is highly reproducible and quantitative; a single internal reference is sufficient for absolute metabolite quantitation over an incredible dynamic range since signals in NMR have the same sensitivity, independent of the properties of the metabolite; (2) a combination of NMR techniques enables the unambiguous identification of structures for unknown metabolites, which is important considering that a majority of the detected metabolites in complex biological mixtures is unknown; (3) NMR enables analysis of intact biofluids and tissues with minimal or no need for sample separation or preparation, which is important considering that factors associated with sample preparation and separation contribute to analytical variability and represent a major bottleneck; (4) NMR is nondestructive, which means the sample remains intact after the analysis and can be used for reanalysis at a later time period or analysis using other methods such as MS; (5) NMR possesses unsurpassed capability to trace metabolic pathways and measure metabolic fluxes utilizing isotope-labeled substrates; (6) NMR provides unique opportunities to translate in vitro findings to clinical applications in vivo; and, finally, (7) metabolite profiles obtained by NMR are virtually independent of the operator and instrument used, which provides a high degree of reliability to the derived results. A variety of procedures have been reported for sample preparation depending on the type of the study. Sample requirements are typically 100−200 μL for biofluids and 5−10 mg for tissue, which are much higher than for MS analysis. With advanced high-throughput NMR methodology, up to ∼200 samples can be measured within a day with the assistance of flow-injection probes and automated liquid handlers [80,81].

^{1}H signals from water protons are very large and thus require suppression, which is typically performed by the application of an appropriate water suppression pulse sequence such as presaturation (PRESAT) [82] or other sequences. Once the water signal has been suppressed, a significant number of metabolites can then be detected in the biological samples. However, the NMR data from biological specimens contain a large number of peaks which often overlap. As a result, metabolites of lower concentration are frequently overshadowed by larger peaks, thereby limiting the detection capabilities of the instrument. Typically, 50−200 metabolites can be detected, depending on the type of sample, with urine providing the highest number. In order to reduce problems with signal overlap that are encountered in 1D NMR experiments, 2D NMR spectroscopy can be used to assist in the identification of putative metabolites found in the 1D NMR spectrum. 2D NMR spectroscopy is less commonly employed in metabolic profiling approaches due to its longer acquisition time and more involved data analysis requirements. The high reproducibility and quantitative abilities of NMR, its use in combination with multivariate statistical methods, make it a very powerful approach to identifying putative metabolites from a complex spectrum. NMR continues to witness widespread use in metabolomics.

NMR's ability to analyze intact samples is fully exploited for the analysis of samples such as urine and saliva that are usually devoid of large amount of interfering proteins. Traditionally, even widely used samples such as serum/plasma are analyzed using intact samples by suppressing protein signals using the Carr−Purcell−Mieboom−Gill NMR sequence [45]. However, it is increasingly realized that analysis of blood serum/plasma by NMR is improved by physically removing serum/plasma proteins before the analysis. Broadly, two approaches are used for removal of proteins: one is ultrafiltration and the other, protein precipitation using an organic solvent such as methanol, acetonitrile, or others [48,83]. More recently, it was shown that protein precipitation using methanol (2:1 v/v) provides an efficient route to remove proteins and recover metabolites quantitatively for improved metabolomics analysis of serum/plasma by NMR [84,85].

F Global Versus Targeted Metabolic Profiling

Metabolomics-based studies can be divided into two main categories: targeted and nontargeted metabolic profiling approaches [86]. Untargeted, or "global," metabolic profiling studies are "hypothesis generating" and have mostly focused on the discovery of new metabolite biomarkers or new alterations in metabolism caused by biological stresses. Targeted approaches have focused more on metabolite-specific studies, as well as for the validation of putative biomarkers.

While it is likely impossible to completely profile all metabolites in a complex biological sample, current analytical methods are quite capable of measuring hundreds to a few thousand metabolites, especially when several platforms are combined. Initially, metabolomics investigations primarily used global metabolic profiling methods involving the analysis of all detectable signals, including both known and unidentified metabolite peaks. Very often, metabolites are not identified prior to analysis. Instead, the complex data are subjected to statistical analysis (discussed later), after a preprocessing step. For NMR, preprocessing includes apodization and zero-filling, Fourier transformation, phase and baseline correction, peak alignment, solvent peak removal, and (optionally) data binning [87]. For MS, spectral peak quantitation and alignment are typically followed by metabolite identification, wherever possible. Typically, only one-third of metabolite identities are assigned with confidence in global profiling studies. For LC-MS, preprocessing also includes the removal of isotope and adduct peaks resulting in simplified and better quantified spectra. Subsequently, metabolite features that distinguish sample classes are identified and then the structures of distinguishing metabolic features are established wherever possible.

In targeted metabolite profiling, a set of known metabolites are quantitated. The identities of metabolites are established a priori based on available databases and using standard compounds; the identified metabolite peaks are then quantified relatively, or sometimes absolutely based on the inclusion of internal or external reference compounds. The resulting data can then be used for pathway analysis to prove or disprove biological hypotheses, or as input variables for statistical analysis. Because of the reliable peak identification and measurement of metabolite intensities or even concentrations, targeted metabolomics provides greater insights into the dynamics and fluxes of metabolites and promises more robust statistical models for distinguishing sample classes with better classification accuracy. As NMR spectroscopy already provides highly reproducible results with a coefficient of variation (CV) of 2−3%, targeted metabolomics can be performed easily using NMR [52]. Targeted metabolomics using tandem mass spectrometry (LC-MS/MS) enables fast MS data acquisition resulting in quantitative data with reasonably good reproducibility (5−10% average CV) [88,89]. A variety of internal standardization methods using isotope-labeled compounds (^{2}H, ^{13}C, ^{15}N) as well as external standardization methods are also available to provide much better reproducibility. The inclusion of internal standards reduces these CVs to $<5\%$.

Targeted metabolomics analysis encompasses the measurement and identification of a specific list of metabolites from selected pathways of interest [90]. This approach can be used for pharmacokinetic studies, drug metabolism, and for the validation of putative markers in numerous disorders [2,91,92]. The main strengths of this approach are its quantitative capabilities and reliability. However, one limitation is that it requires knowledge of the metabolite's identity, and as a result novel biological markers can be difficult to discover.

While multiple MS techniques can be applied, the use of triple quadrupole MS coupled with chromatographic techniques has become the standard approach used for targeted metabolomics analyses of a number of compound classes including amino acids, organic acids, carbohydrates, amines, and nucleotides [86]. Currently, the detection of 100−300 targeted metabolites is possible using multiple reaction monitoring [80,86].

IV DATA ANALYSIS

In addition to the analytical methods, advanced statistical and typically multivariate methods play an essential role in metabolite profiling [93,94]. While univariate methods such as the Student's *t*-test or *p*-value calculations are extremely useful in identifying individual metabolite changes or biomarker candidates, it has been recognized for some time that *individually* almost all metabolite biomarkers are likely to be insufficient in terms of performance. Thus, in order to build predictive models based on multiple biomarkers that can improve performance, multivariate statistical methods are employed. Multivariate methods are also very useful for reducing the dimensionality of the NMR/MS data, and to extract the maximum information. Multivariate methods are generally capable of processing several thousand inputs or "variables" and their corresponding intensities; however, most practical applications typically involve a dozen or less biomarkers because of the increased effort needed to develop and validate each marker individually.

A Data Pretreatment

Prior to data analysis, there are a number of data preprocessing steps that are often necessary in order to extract the optimal information from the complex biological data. These steps include normalization and scaling, along with additional preprocessing steps such as noise reduction, baseline correction, peak deconvolution, and peak alignment. These steps ensure that valuable information is derived from the acquired data with only minimal bias. The specific treatment, however, depends on the analytical platform used to acquire the data.

For NMR spectra, baseline correction is performed to reduce offsets that might introduce errors into the accurate measurements of metabolites [95]. Spectral data alignment is performed using the peak from a reference

compound such as TSP (trimethylsilyl propionate-d_4 sodium salt) or DSS (sodium 4,4-dimethyl-4-silapentane-1-sulphonate-d_6). On the other hand, mass spectra are normally aligned based on retention times (RT) or mass/charge ratios in order to counteract column or instrument drift over time. In LC-MS, Quantitative Structure−Retention Relationship modeling can be used to predict RT [96]. For GC-MS analysis, retention time locking procedures along with a library of fatty acid methyl esters (FAMEs) with different chain lengths that are added to each biological sample provide an opportunity to circumvent problems associated with column drift [97]. These procedures have also facilitated the identification of metabolites irrespective of the specific GC-MS platform used in the analysis.

Data normalization is carried out to minimize the effects of unwanted variations that arise as a result of different sample volumes, concentrations, and instrumental sensitivity changes. There are various types of normalization that are applied, such as total (spectral) sum normalization or normalization to a reference compound. Methyl succinate or d_{27}-myristic acid is used for GC-MS, TSP normalization is used for NMR studies, while more generally creatinine normalization is used for urine analysis [98]. Scaling is sometime performed in order to compare or enhance metabolites that are present at low concentrations. This is made necessary by virtue of the fact that, when performing multivariate data analysis, low abundance metabolites can be misinterpreted or even excluded. A number of different scaling methods are used in metabolomics including variance scaling, autoscaling, and Pareto scaling. In addition, log transformation of the data can be used to reduce data skew and outliers.

B Univariate Analysis

One of the simplest forms of statistical analysis employed in metabolic profiling studies, univariate analysis involves a single variable at a time, and is quite commonly used in the first stages of systems biology research and biomarker discovery. Univariate analysis is also used to further validate the performance of single putative metabolite biomarkers. There are various univariate statistical methods that are commonly used in metabolomics, including the Student's *t*-test, the Mann−Whitney U-test, and the receiver operating characteristic (ROC) curve [99]. The Student's *t*-test is widely used since it gives the probability that two sample sets are distinguishable for a particular variable (metabolite). However, it does assume that the data are normally distributed, which may not always be the case in metabolomics data sets. Alternatively, the U-test does not require normally distributed data.

ROC curves are often used to evaluate the performance of a diagnostic test or the potential of putative biomarkers [100]. As shown in Fig. 5.2A, the ROC curve is a plot of sensitivity (true positive rate) versus 1-specificity (false positive rate). In an ROC curve at the (0,0) coordinate, all test results are negative while at the (1,1) coordinate all results are positive. The diagonal line connecting the two coordinates indicates that the diagnostic test results are purely by chance (random prediction) and, therefore, that the test lacks diagnostic capabilities. The overall utility of a diagnostic test is often assessed by calculating the area under the ROC (AUROC) curve. In these cases, an area of 1 represents a perfect test while an AUROC of 0.5 corresponds to the diagonal line indicating a random prediction. A main advantage of the ROC curve is the ability to see the trade-off between decreasing the sensitivity and increasing the specificity and vice versa, which is crucial when performing clinical studies.

C Multivariate Statistical Analysis

In general, multivariate statistical approaches are broadly classified into two categories: "unsupervised" and "supervised" methods. In unsupervised analysis, the sample status (e.g., "case" or "control") is not known or used during the statistical analysis, whereas in supervised methods this information is part of the analysis.

D Unsupervised Statistical Methods

Several different types of unsupervised methods are used in metabolomics, including principal component analysis (PCA), hierarchical cluster analysis (HCA), k-nearest neighbor, and factor analysis [102]. Many of these methods are used in exploratory data analysis for hypothesis generation.

PCA (see Fig. 5.2B) is the most commonly used unsupervised method in metabolomics [99]. It is a powerful data reduction method and also serves as a useful starting point in multivariate statistical data analysis. PCA is commonly applied to detect possible outliers and clusters present in the sample set. PCA is a mathematical procedure that transforms the data set into a set of ranked principal components (PCs) that explain the variance in the data. As a result, the transformed data matrix is described as the product of a set of "scores" based on the PCs and corresponding "loadings" which give the contribution of each metabolite to that particular PC. The first PC explains the direction that describes the largest variance in the metabolite signals; this is followed by the second and subsequent PCs, which explain successively lower variance. The loadings provide information on the variables (metabolite signals) that account for separation of the data clusters in the scores plot. Since no prior knowledge of the sample class (control,

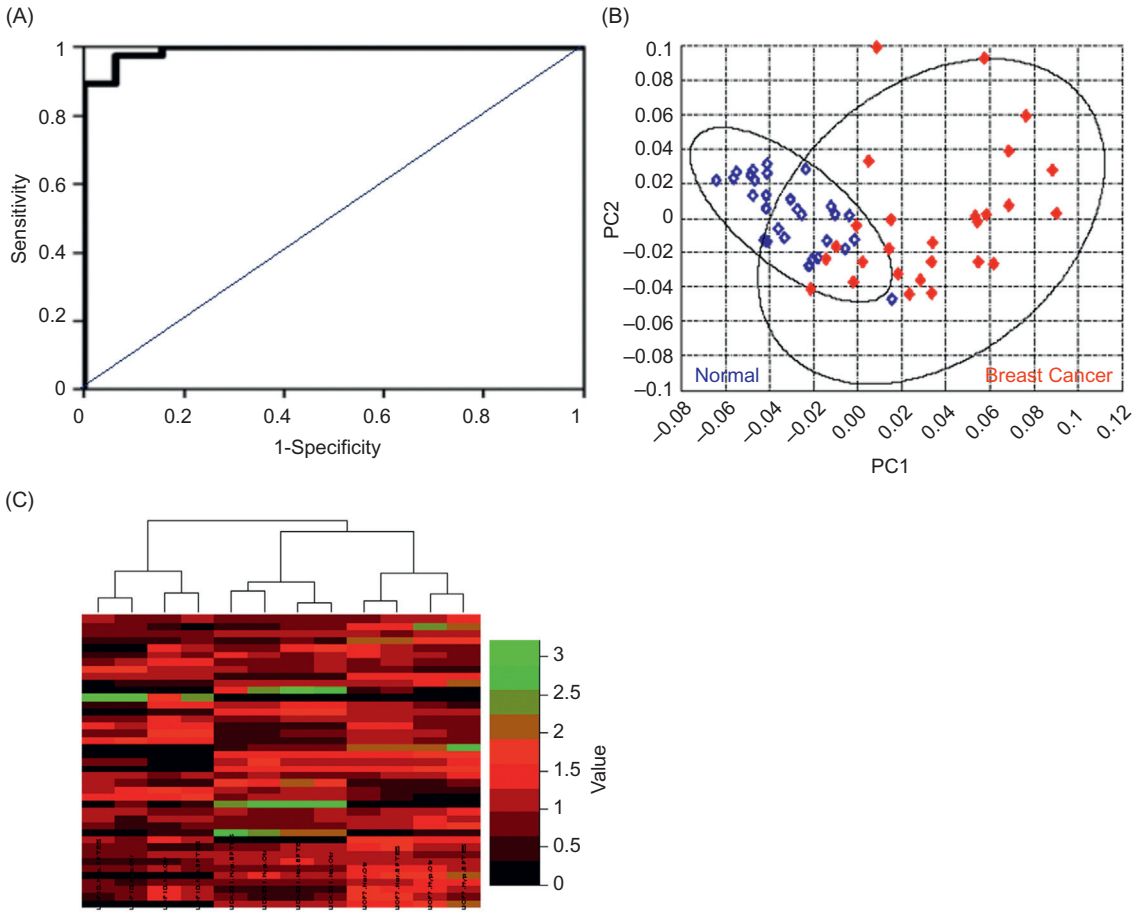

FIGURE 5.2 (A) ROC curve for a 12-metabolite NMR-based statistical model for detecting pancreatic cancer; (B) Score plots from the results of PCA analysis of NMR spectra from breast cancer and normal controls. Open diamonds represent normal samples and black solid diamonds represent breast cancer samples. Ellipses in the score plots illustrate the 95% confidence level; and (C) Typical results of a HCA of metabolic profiles from three types of cells with and without treatment with a drug. *Part (A) modified from [101] K. Owusu-Sarfo, V.M. Asiago, L. Deng, H. Gu, S. Wei, N. Shanaiah, et al., NMR-based metabolite profiling of pancreatic cancer. Curr. Metabolomics 2 (2014) 204–212 and Part (B) modified from H. Gu, Z. Pan, B. Xi, V. Asiago, B. Musselman, D. Raftery, Principal component directed partial least squares analysis for combining nuclear magnetic resonance and mass spectrometry data in metabolomics: application to the detection of breast cancer. Anal. Chim. Acta. 686 (2011) 57–63.*

disease, etc.) is utilized, the variables identified from the corresponding loading plots may serve as potential biomarkers for further (confirmatory) analysis [103]. However, cluster separation may also result from confounding effects (age, gender, BMI, etc.) unrelated to the condition under study.

HCA seeks to define natural clusters based on comparing distances between pairs of samples or variables within the data set. It is most useful when the intention is to cluster a small number of variables. The distances between all samples are calculated, and the smallest distances between samples imply that this subset of samples share similar metabolite levels, thereby representing similar physiological properties or disease states or stages for example. The process is repeated until all samples in the data set are grouped. HCA is often used in combination with heat maps to help visualize patterns in the data (see Fig. 5.2C). In biomarker discovery, HCA is usually used as a supporting method for more commonly used unsupervised methods such as PCA [104].

E Supervised Statistical Methods

Most predictive models in metabolomics currently rely on supervised statistical analysis in order to reduce the effects of confounding factors such as age, diet, race, gender, etc., which can affect the performance of the model. Supervised methods require a training data set, in which the outcome (i.e., disease or healthy) is known, to build a predictive model. Typically, sample data sets

are split into training and test sets, although some practitioners utilize an intermediate set of data that is used to improve the modeling. After training, the model's accuracy is evaluated by classifying samples in the test set of samples. Typically, cross-validation [94] is used to test the robustness of putative biomarker candidates during the training process as well as to identify the best model, given the training set of sample data.

Two of the most popular methods for supervised pattern recognition include partial least squares discriminant analysis (PLS-DA) [105] (which is often combined with orthogonal signal correction [106]) and logistic regression (LR). Other, often used, methods include soft independent modeling of class analogies, genetic programming, and neural networks.

PLS is a regression algorithm that predicts a vector Y, the dependent variable(s) from the independent data in matrix X, which is typically the NMR or MS data. PLS finds a combination of the X data that explains the maximum variance in the Y data. In PLS-DA, the Y-matrix contains categorical class variables, for example, 1 = cancer; 0 = control [107]. Similar to PCA, where the PCs describe the variance, each orthogonal axis in PLS-DA is referred to as a latent variable (LV). The LVs contain the weight of each variable (metabolite signal). By maximizing the differences between two selected classes, putative biomarkers can be identified from PLS-DA from the weights of the LV. While PLS-DA statistical models can establish the difference between preassigned sample groups, they can often over-fit the data. One must, therefore, carefully validate the results of the model established during training, and use prudence when applying the results of PLS-DA to new data sets [108].

LR is a commonly used method to predict the probability of an event (toxicity, disease, effect of a drug) from a set of predictor variables that can be a variety of inputs, either numerical or categorical. The output, or dependent variable, itself is categorical (i.e., cancer vs normal). In LR, the occurrence of an event is mathematically evaluated in terms of its probability, which can be summarized in the equation below:

$$\text{Logit}\,(p) = \ln(p_i/1-p) = \beta_0 + \beta_1 x_1 + \cdots + \beta_k x_k$$

where p is the probability of an event, x_i are input variables, β_0 is the intercept of the regression line, and β_k are fit parameters. The output of LR analysis is a set of predictors indicated by the nonzero β coefficients, which can be useful in determining the most important variable(s) for statistical modeling. In metabolomics, LR is most often used to combine a limited number of biomarker candidates as a predictive algorithm for diagnostic, prognostic, or other purposes.

In general, it is extremely important to validate the findings of any of these multivariate methods (including unsupervised methods) using extensive cross-validation [109] and, in particular, additional sets of samples (preferably blinded and from other locations) and which are sufficiently large to yield statistically significant results. Beyond the statistical aspects of validation, ultimately, biological validation, such as involving a disease hypothesis specifically related to the discovered biomarkers, may be required before acceptance by the medical and scientific communities can be anticipated.

F Correlation and Other Associative Approaches

In addition to regression and clustering analyses, correlation approaches have attracted major interest in metabolomics for their ability to identify relationships among different metabolites and/or pathways, to reduce spectral complexity and help identify unknown metabolite structures. In addition to standard correlation calculations such as the Pearson correlation between two metabolites, several new and alternative approaches have been introduced. For example, STOCSY (statistical total correlation spectroscopy) is a method applied to 1D NMR spectra to generate correlation coefficients between every pair of 1D NMR peaks across multiple spectra. Peaks showing high correlations provide clues to unknown metabolite identification [110]. A large number of variants of STOCSY have been developed, such as subset optimization by reference matching, which improves data quality and peak alignment using correlations across subsets of samples [111], or statistical heterospectroscopy, which correlates peaks in MS with NMR for improved unknown identification and biomarker discovery [112].

A novel statistical approach based on peak ratios was introduced for the analysis of NMR and mass spectral data: RANSY (Ratio Analysis of NMR Spectroscopy) was demonstrated to isolate peaks from metabolites in complex mixtures based on the principle that the peak intensity or integral ratios between peaks from the same metabolite are fixed [113]. The RANSY spectrum for individual metabolite is obtained by generating peak ratios that are divided by the ratios' standard deviation σ across a set of small number (viz., 8−10) of spectra. NMR peaks arising from the same molecule exhibit fixed ratios, which leads to small σ and thus provides large RANSY values. This ratio analysis approach was extended to identify metabolites using mass spectral data [114], in a method called RAMSY (Ratio Analysis of Mass Spectrometry) that efficiently isolates mass fragment peaks for the same metabolite using a single mass chromatogram.

G Major Challenge: Unknown Metabolite Identification

The powerful methods of NMR and MS provide capabilities to measure hundreds of metabolites. However, the current peak identification capabilities in MS are limited to approximately one-third of globally detected metabolite features, which are typically assigned based on databases of spectra for authentic compounds. Identifying unknown metabolites is thus a challenging endeavor and a major focus in the metabolomics field. MS methodologies to identify unknown metabolites mostly rely on combinations of high-resolution MS to determine the chemical formula along with MS/MS experiments to determine fragmentation patterns, along with general rules of chemical bonding [115]. NMR analysis using an array of 1D/2D experiments represents a powerful approach for unknown identification de novo. Using this approach, nearly 70 metabolites have been identified and quantified in the NMR spectra of blood [84]. However, unambiguous assignment of peaks for many metabolites with low (less than or equal to few micromolar) concentrations is especially challenging, since many of these peaks are often overlapped with abundant metabolite signals.

Unknown metabolite identification in the spectra of human urine is generally more challenging than other biological mixtures. The significant amount of salt present in urine makes it less attractive for analysis using LC-MS, and GC-MS is more often used for analysis. Urine spectra generally provide significantly higher numbers of signals compared to serum/plasma, and hence urine is a rich source of information specifically for investigations of biomarkers of food and nutrition. Unlike serum/plasma, urine metabolic profiles are sensitive to contributions from numerous factors including diet, medications, personal habits such as physical activity or smoking, gender, age, gut microbe diversity, and genetics [49]. Because of these factors, urine from even the same individual exhibits significant variability depending on the time of the day the urine was collected [116]. Urine pH also varies significantly (from $\sim$5 to 8), which together with the high salt concentration causes significant peak shifts for many metabolites when analyzing by NMR. Nevertheless, significant improvements in the identification of unknown metabolites in urine have been recently achieved based on comprehensive analysis using new database software and spiking with authentic compounds [117]. Interestingly, the number of urine metabolites identified by NMR is higher than that obtained from the more highly sensitive GC-MS.

H Mechanistic Studies—Flux Analysis

Most metabolomics analyses measure relative changes in abundance or concentrations for different sample groups. However, a limitation of the analysis of metabolites at steady state is that their concentrations may lack sufficiently specific information to obtain an unambiguous understanding of the biosynthesis of metabolites. This situation occurs because many metabolites are associated with multiple metabolic pathways. Additionally, in cells and tissues many metabolic levels are controlled by regulation through homeostasis. Hence, the contribution of each metabolite to a specific pathway is masked. Thus it has been increasingly realized that a metabolic pathway-focused, hypothesis testing approach is desirable to further understand pathogenesis and potentially to discover additional disease biomarkers. An approach that promises more specific information on metabolic pathways involves the incorporation of stable isotopes into the downstream metabolites using precursors enriched with stable isotopes such as ^{2}H, ^{13}C, or ^{15}N. This approach provides vital clues regarding biological mechanisms by tracing isotope-enriched metabolite products and long-lived intermediates that are produced. Flux analysis allows a better understanding of the dynamics of metabolic pathways and enables better modeling of intracellular metabolite levels and determination of the rates at which metabolites are produced or consumed. More specifically, metabolite profiling of cells supplied with ^{13}C-labeled glucose, for example, facilitates the determination of the glucose consumption rate as well as the rates at which the downstream metabolic products of glycolysis are produced. As an example, ^{13}C-labeled lactate produced from the glycolysis of ^{13}C-glucose in cancer cells can be distinguished from the same lactate produced from other pathways based on the presence or absence of the embedded ^{13}C isotope [118]. Advances in targeted metabolic profiling methods have improved the accuracy of metabolite quantitation and facilitated increased interest in understanding diseases based on altered metabolic pathways. Both NMR and MS methods are used in stable isotope-based flux analysis using labeled precursors.

V APPLICATIONS TO FOOD AND NUTRITION

It is well known that foods and nutrients as well as dietary patterns interact with metabolic processes and contribute to a reduction or an increase in the risk of a number of important diseases [119]. Thus, diet plays a major role in human health and also constitutes an important risk factor for many diseases. A large number of metabolomics investigations are therefore focused on understating the composition of foods as well as diet-induced metabolic changes in biological specimens such as urine and blood [120].

Most foods are essentially mixtures of metabolites and other compounds in solid or liquid forms that are similar

to biofluids in complexity. Metabolomics provides a powerful means for investigations of foods; nevertheless, the comprehensive analysis including quantitative measurements of ingredient micronutrients is still lacking for many foods. To date, studies on numerous fruits, spices, oils, and beverages have been reported, with NMR as the major method for quantitative characterization of polar metabolites with relatively high abundances [121]. As an example, metabolomics is used to characterize wine to prevent adulteration, classify wines according to variety, region, and vintage, and to understand the fermentation behavior, using NMR spectroscopy [122–124]. Metabolite profiles of beers from different sites, including different countries, produced under various fermentation conditions, have also been compared. Significant differences in metabolite composition observed among the various types suggest the utility of the approach for controlling the quality and identifying fermentation conditions [125–127]. A study focused on the analysis of honey using an advanced approach to identify low concentration metabolites that differentiated the honey's origin by reducing the effect of large, uninteresting sugar signals [128]. Metabolomics methods have also been applied to coffee produced by different manufacturers [129]. Based on multivariate analysis of the ^{1}H NMR data, 99% of the samples were correctly attributed to their manufacturers. Investigations of tea have also been made to discriminate different teas according to the country of origin or to various quality factors. By comparing teas from different countries, it was shown that the concentration of metabolites such as catechin and theanine can be used to classify fermentation and processing of tea [130,131]. Numerous components of tea including theogallin (5-galloyl quinic acid), theobromine, 2-*O*-(beta-L-arabinopyranosyl)-myo-inositol, fatty acids, sucrose, and some minor sugar-containing compounds have also been shown to distinguish different tea types [131].

Identification of dietary biomarkers is also of significant interest to evaluate their impact on nutritional value and adverse effects on health. Broadly, dietary biomarkers are grouped under two major categories: recovery biomarkers and concentration biomarkers. Recovery biomarkers signify absolute dietary intake over a certain period of time; the amount of nitrogen in 24-hour urine is an example of a recovery biomarker. On the other hand, concentration biomarkers that correlate with diet do not represent the absolute level of dietary intake; vitamin E in plasma, for example, represents a concentration biomarker [132]. Current methods that use food frequency questionnaires (FFQs) with 24-hour recalls and weights of the food are often prone to errors. Therefore, the biomarker studies are challenged by the insufficient precision associated with the detection of diet–disease associations based on FFQs.

Metabolomics promises numerous potential benefits to alleviate some of the challenges in conventional methods of dietary assessment through the identification of nutritional biomarkers. Dietary effects are reflected in variations of metabolite profiles and specific effects can be detected by measuring metabolites in biofluids [133]. An advantage of metabolomics for nutritional studies is that a large number of dietary markers can be investigated in a single measurement. Owing to these potential benefits, the field has witnessed a large increase in applications over the last five years. Currently, three different approaches are used for the discovery of dietary biomarkers: (a) intervention studies, where participants are made to consume a specific food, (b) cohort studies, where groups of consumers and nonconsumers of a specific food are compared, and (c) dietary pattern studies, where the analysis focuses on certain dietary patterns. A recent article has reviewed these approaches in greater detail [120].

Intervention studies have resulted in the identification of a number of potential dietary biomarkers. Among them, identification of proline betaine from citrus fruit intake is an important finding [134]. A number of independent studies have validated this biomarker [135,136]. Many studies have focused on the identification of food sources of various biomarkers found in complex meals and representative dietary patterns. Using sensitivity and specificity analyses many conjugated isothiocyanates have been identified as biomarkers of brassica intake [137]; however, only 23% of these biomarkers could be validated, which highlights the need to use proper study protocols for reliable identification and validation [138]. Other intervention studies have identified biomarkers for meat and fish [139–141]. Different effects of meat and fish on the plasma metabolome have also been investigated. For example, a beef meal showed higher concentrations of 2-aminoadipic acid, β-alanine, and 4-hydroxyproline compared with a baked herring meal [142]. Other studies have shown that a daily consumption of 40 g of dark chocolate for 2 weeks altered the metabolic profile involving lactate, citrate, succinate, *trans*-aconitate, urea, proline, adrenaline, 3,4-dihydroxyphenylalanine (DOPA), 3-methoxy-tyrosine, methylamines, *p*-cresol sulfate, and hippurate in healthy subjects [143].

Many cohort studies have also been focused on the identification of putative biomarkers. Unlike intervention studies, cohort studies make use of self-reported dietary data for searching biomarkers. A study of prostate, lung, colorectal, and ovarian cancer screening compared the serum metabolite profile with dietary data collected using FFQ and detected 39 metabolites that were correlated with 13 dietary groups [144]. Notable among these were correlations of citrus intake with stachydrine, chiro-inositol, scyllo-inositol, and *N*-methyl proline; fish intake with 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid,

docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA); peanut intake with tryptophan betaine and 4-vinylphenol sulfate, and coffee intake with trigonelline-*N*-methylnicotinate and quinate. However, a number of metabolites were not specific to food; for example, DHA correlated with fish and rice. Hence, validation and additional studies based on intervention are required for more insights into these biomarkers. Separately, a large cohort study ($n = 2047$) identified ferritin, glycine, 4 diacyl phosphatidylcholines, 11 acylalkyl phosphatidylcholines, 2 lysophosphatidylcholines, and 2 sphingomyelins in serum as associated with red meat consumption; six of these were found to be associated with diabetes risk [145]. A downside of this interesting study, however, is that the dietary information relied on the estimates of meat consumption by FFQ and that no distinction was made between processed and unprocessed meat. Another cohort study based on metabolic profiling of urine identified higher concentrations of benzoxazinoids and alkylresorcinol metabolites as well as many gut microbial metabolites, such as enterolactones, hydroxybenzoic and dihydroferulic acid in bread consumers. In whole-grain bread consumers, 2,8-dihydroxyquinoline glucuronide was found to be higher [146].

Numerous studies report the discovery of dietary biomarkers based on dietary patterns [120]. For example, based on statistical analysis of three dietary patterns and self-reported data, a number of metabolites that are reflective of specific food intake were identified [147]. Another study identified lipid patterns that are predictive of dietary fat intake, alcohol intake, or fish intake [148]; this study identified lysophosphatidylcholine as a potential biomarker of alcohol consumption and lysophosphatidylethanolamine and phosphatidylethanolamine diacyl of fish intake. More recently, a study was made using urine metabolomics for the development of a model for compliance measures using two dietary patterns, a Nordic diet or an average Danish diet. This study provided a robust model with a misclassification rate of 19%, which highlights the utility of this approach to evaluate the compliance measures [149]. The application of metabolomics to nutrition studies continues to advance at a fast pace.

VI SUMMARY

Through its ability to simultaneously analyze hundreds of metabolites, metabolomics approaches promise numerous avenues for the establishment of multiple biomarkers that correlate with food and nutrition as well as to provide detailed information on biological states, diseases, and health risks. The discovery of dietary biomarkers offers new avenues for establishing beneficial or deleterious effects of various diets on human health. In view of its importance in these areas, the metabolomics field is witnessing an exponential growth of applications to food and nutrition. As a consequence, an increasing number of biomarkers have been detected based on metabolomics studies of dietary interventions, cohorts as well as dietary patterns. These identified biomarkers, however, need proper validation before they can be considered for translational investigations. Validation is critical and also challenging due to numerous factors that confound the metabolite data. On the metabolomics technology side, the unsolved challenge is the lack of knowledge of identities for a large fraction of the detected metabolites. Further, continuously increasing advances in analytical technologies have enabled detection of an increasing number of metabolites from the same biological samples; thus, the advances in analytical techniques also continuously increase the fraction of unidentified metabolites. In view of this, a major focus in the metabolomics field is on developing methods for unknown metabolite identification and expanding pool of quantifiable metabolites. Such advances combined with improvements to study protocols that eliminate confounding effects are anticipated to impact immensely the discovery and validation of dietary biomarkers and their use in nutritional intervention.

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Chapter 6

Translational Research: Concepts and Methods in Dissemination and Implementation Research

Jamie M. Zoellner and Kathleen J. Porter
University of Virginia, Charlottesville, VA, United States

I INTRODUCTION

Scientific discoveries are not efficiently translated into everyday practice to benefit patient and population health. The lag time for scientific discoveries to be integrated into clinical and community practices has been estimated to take about 17 years [1]. Of further concern, it is estimated that only about 14% of research discoveries are translated to benefit patient care in this timeframe. Additionally, due to the complexities of moving scientific discoveries along the research pipeline to establish widespread clinical and population impact, others argue that this lag time is much longer [2]. Despite lack of clear consensus on the number of years, there is broad agreement that the research pipeline process is entirely too long [3]. As a result, translational research and translation science have emerged as a key research paradigm for accelerating scientific discovery into patient and population benefit.

Translation is the process of turning observations in the laboratory, clinic, and community into interventions that improve the health of individuals and the public [4]. Translational science is the field of investigation focused on understanding the scientific and operational principles underlying each step of the translational process [4]. Therefore, research within translational science involves a bidirectional process that moves among basic, clinical, practice, population, and policy-based research and requires multidisciplinary team. Across different fields, there are a variety of models that depict the phases of the translational research process, ranging from two to five stages [5,6]. For example, the National Institutes of Health (NIH) describes translational research in two defined stages, defining T1 as "*the process of applying discoveries generated during research in the laboratory, and in preclinical studies, to the development of trials and studies in humans*" and T2 as "*the research aimed at enhancing the adoption of best practices in the community*." Others have described the translation spectrum in more distinct phases, including T1−T3 [6,7], T1−T4 [8], and T0−T4 [9,10] descriptions of translation. Table 6.1 provides an overview of the T0−T4 translational research phases along with examples of nutrition research associated with each phase. In this five-stage model, T0−T2 involves the translation of basic science to human studies while T3−T4 addresses the translation of new information into the clinic and community. Regardless of operationalization challenges related to defining the stages of translational research process, there is broad acceptance and agreement on the importance of translational research. Furthermore, the overarching concept of translational research is consistent.

Being aware of differences in taxonomy across prominent models of translation research is key to promoting effective communication of translation research concepts within a multidisciplinary team. In this chapter, the T0−T4 descriptors and research phases, as illustrated in Table 6.1, will be used.

This chapter provides an overview of key concepts, methods, and trial types in translational research. The primary purpose of this chapter is to focus on the later phases of translational research, or the translation of research aimed at the promotion and the adoption of the

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TABLE 6.1 Translational Research Phases, Example Types of Research, and Sample Research Questions

Research Phase	Example Types of Research	Sample Research Question
T0: Basic and applied science research	Defining mechanisms and targets: basic research, animal research, preclinical and preintervention studies	What are the mechanistic actions of carbohydrate sources on liver and pancreatic tissues in an experimental rat model?
T1: Translation to humans	Discovery of application to human health: proof of concept, early stage human clinical trials	What are the mechanistic actions of ingested carbohydrate sources on liver and pancreatic tissues in humans?
T2: Translational to patients	Discovery of application to clinical settings: later stage human clinical trials, efficacy studies, controlled observational studies, clinical guidelines	What is the effect of regulating the amount of carbohydrate sources among prediabetic subjects in a highly controlled clinical research setting?
T3: Translation to practice	Health application of evidence-based practice guidelines and practice guidelines to health practice: effectiveness research, CER, D&I research, health services research	What is the relative effect on diabetic risk factors (e.g., A1c, fasting glucose, weight) among prediabetic patients in free-living conditions when randomized to a 6-month diet which regulates carbohydrates versus a 6-month usual diet comparison condition?
T4: Translation to community	Health application of practice to population health impact: D&I research, scale-up and spread research, population health impact	What is the degree to which nutrition professionals in community health clinics can adopt, implement, and maintain an evidence-based program to encourage healthy carbohydrate modifications among at-risk clients?

best evidence-based practices into real-world clinical and community settings. Specifically, this chapter concentrates on concepts and application of dissemination and implementation (D&I), an emerging and critical field of research [3]. Dissemination research is defined as an active research approach of spreading evidence-based interventions to a targeted audience via determined channels using planned strategies and examining the success of this dissemination. Implementation research is the process of putting to use or integrating evidence-based interventions within a setting and examining whether the interventions are put into place as designed and thus focusing on the adoption or uptake of clinical interventions by providers and/or systems of care. This chapter will emphasize methodological considerations and illustrates common models and measures used in D&I research. Illustrated research concepts and resources are relevant for nutrition researchers and practitioners.

II KEY CONCEPTS IN TRANSLATIONAL RESEARCH

An overview of key terms and definitions relevant to translational research is presented in Table 6.2. First, it is important to define and understand internal and external validities. Internal validity refers to the degree to which study bias is minimized and a causal relationship of the treatment/clinical intervention within a study sample can be estimated [11]. When considering internal validity, the focus is on eliminating confounding variables so that a cause and effect relationship can be established between independent and dependent variables. Alternatively, external validity refers to the degree to which research findings from a study can be generalized to other populations and settings [11]. External validity is focused on conducting the research with a study population that represents the target audience and in more realistic settings where control variables are not artificially contrived. While both forms of validity are important throughout the spectrum of translational research process, more emphasis is generally placed on internal validity in the earlier stages and on external validity in the later phases. Notably, these forms of validity are not all-or-none or black-and-white, and they can vary widely along the research continuum. Researchers and practitioners need to carefully consider and be transparent in reporting the methodological factors that influence both the internal and external validity of research findings.

Second, application of translational research concepts demands awareness of the types of research associated with the different stages in translation research process. Across the T0–T4 spectrum, there are distinct types of research at each stage: preintervention research (T0/T1); efficacy research (T2); effectiveness research (T3); D&I research (T3/T4) [3]; and scale-up and spread research (T4) [3,12].

TABLE 6.2 Key Concepts, Methods, and Trial Types in Translational Research

Term	Definition
Internal validity [11]	The degree to which study bias is minimized and a causal relationship of the treatment/clinical intervention within a study sample can be estimated.
	Example: *Can effect sizes from a randomized-controlled weight loss study (e.g., magnitude of weight change between a treatment group and control group) be determined, while minimizing confounding variable?*
External validity [11]	The degree to which research findings from a study can be generalized to other populations and settings.
	Example: *Can the effects from a weight loss study be generalized to real-world practice settings (e.g., health care clinic, community center), and diverse participants (e.g., minorities, low health literate)?*
Preintervention research	Research that is done in a controlled laboratory setting, typically using nonhuman subjects. The focus is on understanding the cellular and molecular mechanisms that underlie a disease or disease process.
	Example: *Is there an observed relationship between variables?* (T0/T1)
Efficacy research	Highly controlled clinical research that is primarily concerned with internal validity. Threats to causal inference are typically reduced by using homogeneous samples/settings and controlling intervention parameters. The primary outcome measures target individuals and are usually symptom specific.
	Example: *Does the treatment work under optimal conditions?* (T2)
Effectiveness research	Clinical or community research that is primarily concerned with external validity. Threats to generalizability are typically reduced by using heterogeneous samples in "real-world" and diverse study locations. The main outcomes can be broad, ranging from clinical to other individual and organizational-level outcomes (e.g., quality of life, costs).
	Example: *Does the treatment work under real-world conditions?* (T3)
Dissemination research [3]	An active research approach of spreading evidence-based interventions to a targeted audience via determined channels using planned strategies and examining the success of this dissemination. The main outcomes may target individuals and/or organizations, such as awareness, receipt, acceptance, and use of information.
	Example: *Can the treatment be diffused to and engage the targeted users?* (T3/T4)
Implementation research [3]	The process of putting to use or integrating evidence-based interventions within a setting and examining whether the interventions are put into place as designed. Implementation research is focused on the adoption or uptake of clinical interventions by providers and/or systems of care, with primary outcomes such as levels and rates of adoption, fidelity, implementation costs, and sustainability.
	Example: *Can the treatment be adopted by providers and systems?* (T3/T4)
Scale-up and spread research [3,12]	"Deliberate efforts to increase the impact of health service innovations successfully tested in pilot or experimental projects so as to benefit more people and to foster policy and program development on a lasting basis." The main outcomes are similar to D&I outcomes, but typically at national and international levels.
	Example: *Can the treatment be adopted and maintained by a practice-based reach network, for long-term, lasting impact?* (T4)
Hybrid type trial [13]	A study design that takes an a priori focus in the dual assessment of clinical effectiveness and implementation. Hybrid designs can typically take one of three approaches: (1) testing effects of a clinical intervention on relevant outcomes while observing and gathering information on implementation; (2) dual testing of clinical and implementation interventions/strategies; (3) testing of an implementation strategy while observing and gathering information on the clinical intervention's impact on relevant outcomes.
PCTs [3,14,15]	Clinical trials for which the hypothesis and study design are developed specifically to answer the questions faced by decision-makers are called PCTs. The characteristic features of PCTs include: (a) select clinically relevant alternative interventions to compare, (b) include a diverse population of study participants, (c) recruit participants from heterogeneous practice settings, and (d) collect data on a broad range of health outcomes.
CER [16–19]	The conduct and synthesis of research comparing the benefits and harms of different interventions and strategies to prevent, diagnose, treat, and monitor health conditions in real-world settings.

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This progression is shown through the advancement of the sample questions in Tables 6.1 and 6.2: "*Is there an observed relationship between variables?*" (T0/T1) to "*Can the treatment be adopted and maintained by a practice-based reach network, for long-term, lasting impact?*" (T4). These questions illustrate the progression of research inquiry, as well as diversity of trial types and outcomes demanded in translational research.

Third, and of particular relevance to the T3−T4 phases of translational research, recent emphasis has been placed on hybrid type trials [13], pragmatic or practical clinical trials (PCTs) [3,14,15], and comparative effectiveness research (CER) [16−19]. With a focus on efficiency, the primary intent of hybrid type trials is to accelerate the timeline from efficacy trials to public health practices and impact [13]. Hybrid designs blend the study design and methodological elements to dually consider components of both clinical effectiveness and implementation research. There are several contrasting design features between clinical effectiveness and implementation research studies. For example, clinical effectiveness trials typically test the clinical intervention with the unit of randomization and unit of analysis at the patient or clinical unit level. The primary focus is usually on health outcomes with intermediate measures related to process/quality measures typically considered as intermediate. In contrast, implementation trials test an implementation intervention strategy with the unit of randomization and unit of analysis at the provider, clinical unit, or system level. Primary outcomes are usually focused on both the adoption and uptake of the clinical intervention as well as process/quality measures. To address challenges in linking clinical and implementation research designs, Curran and colleagues provide detail of three different hybrid designs [13]. The first design, a hybrid trial type 1, has a primary aim of testing clinical effectiveness on relevant patient-level outcomes and secondary aim relevant to understanding the intervention context and to gathering information about factors that influence implementation processes. The second design, a hybrid trial type 2, equally weights effectiveness and implementation; therefore, there are coprimary aims of simultaneously testing effects of a clinical intervention and feasibility testing of implementation strategies. Finally, a hybrid trial type 3 has a primary aim of testing an implementation strategy and secondary aim of examining the relevant clinical outcomes associated with the implementation trial. Applying hybrid designs require attention to the complexities and balance of internal and external validities and collaboration among a multidisciplinary team [13]. Hybrid designs have been used in nutrition-related studies; notable examples include (1) a two-arm randomized-controlled trial (RCT) hybrid type 1 trial with a primary aim to examine the effects of a behavioral intervention to reduce sugar-sweetened beverage consumption in rural Appalachia [20], (2) a three-arm RCT hybrid type 2 trial with coprimary aims to examine reach, effectiveness, adoption, and cost of the adapted Diabetes Prevention Program (DPP) in a clinical setting [21], and (3) a nonrandomized hybrid type 3 trial with a primary aim to assess implementation of the DPP in three Veterans Affairs (VA) medical centers [22]. The hybrid type 3 trial is further illustrated in Section VI, Examples from the Literature.

PCTs have also gained prominence in the research literature [3,14,15]. PCTs support the clear goal of D&I scholars to shift the research paradigm from a concentrated focus on highly controlled efficacy trials to emphasis on systems and pragmatic approaches [23]. In contrast to efficacy research aimed at testing research questions in optimal conditions, PCTs are designed to answer questions faced by stakeholders and decision-makers in real-world settings, thereby maximizing the external validity of research findings [3,14,15]. By designing research trials with practice needs in mind, PCTs can speed up the translational research findings and accelerate integration into practice and policy [14]. As detailed in Table 6.2, there are a number of notable characteristics and design features of PCTs, including selection of a clinically relevant comparison intervention, inclusion of diverse study participants selected from a variety of practice settings, and assessment measures across a broad range of outcomes. These outcomes should include traditional outcomes, such as changes in patient-level health outcome (e.g., clinical end point, patient reported health behavior and/or psychosocial issue, mortality, and morbidity) as well as outcomes related to quality of life, implementation factors (e.g., costs, fidelity, characteristics of delivery agents, needs of health-care delivery system), and satisfaction (e.g., patient, staff/clinician, organizational decision-makers).

Many of the methodological concepts of PCTs are also relevant when considering CER. While there are a variety of definitions of CER [16−19], one of the most widely accepted is "the conduct and synthesis of research comparing the benefits and harms of different interventions and strategies to prevent, diagnose, treat and monitor health conditions in real-world conditions" [19]. Although CER has historically dominated the biomedical and pharmacological research (i.e., comparison of two different therapeutic options), there has been more recent emphasis on expanding CER to clinical preventative services, behavioral interventions, and public health services delivery systems. One key element of a PCT is selection of a clinically relevant comparison intervention. Historically, when studying research questions related to the efficacy and effectiveness of treatment and intervention options, a placebo, usual care, or noncontact control is used. However, these comparisons are rarely relevant for practice. By definition, CER refers to research that

compares the outcomes of two or more real-world health-care strategies or intervention options that have already been shown to be efficacious. Like PCTs, the intent of CER is to assist patients and community members, clinicians, and stakeholders to make shared decisions on treatment options. A key element of CER is that results inform real-world decision and that they are practical or pragmatic [24]. CER has been used to assess nutrition-related research, including (1) comparison of a counselor-delivered versus web-based-delivered coronary heart disease lifestyle and medication intervention [25,26] and (2) a retrospective cohort study to compare the effectiveness of laparoscopic Roux-en-Y gastric bypass and laparoscopic adjustable gastric banding on weight, adverse health outcomes, and subsequent intervention [27].

Distinguishing among these key concepts, methods, and trial types in translational research is a prerequisite for choosing and applying an appropriate D&I framework. Fortunately, there are a variety of available frameworks to guide the D&I research of nutrition professionals [28].

III COMMON FRAMEWORKS IN D&I RESEARCH

The translation of a discovery into clinical and/or community settings requires that organizations and/or systems change their behaviors by engaging in new organizational practices that permit the adoption, execution, and maintenance of the innovation. Therefore, as with research targeting individual behaviors, there are theories, frameworks, and models. The term "frameworks" will be used in the remainder of this chapter when talking about theories, frameworks, and models that provide researchers and practitioners with theoretical guidance when conducting translational research. These frameworks are most notable in D&I research and attend to the development of motivations and skills needed to adopt, execute, and maintain the innovation at staff and organizational levels. These frameworks guide the development, execution, evaluation, and synthesis of D&I research.

There are numerous D&I frameworks available from which nutrition researchers and practitioners can select; Tabak and colleagues identified 61 different frameworks [28]. While many of these frameworks consist of overlapping constructs, each posits different views of how health-related innovations are disseminated and implemented; focuses on a specific aspect of D&I research; and/or views different potential end goals of D&I research. To add to this complexity, due to these frameworks developing within specific fields, there is a lack of standardized terminology within the frameworks [29], which means that a single construct may have different names across theories. To make the landscape less complicated, D&I scholars have compiled terminology [29] and constructs [30] across frameworks to help standardize language. Other D&I scholars have compiled, organized, and compared frameworks [28,31] to aid nutrition and health professionals and researchers in identifying the framework that is best suited for a given project or research study. Table 6.3 provides a description of six commonly used D&I frameworks and the following section describes their development, focus, and key constructs [29]. Examples of nutrition-related studies that have applied the different frameworks are also included.

A Diffusion of Innovations

Developed by Rogers in the early 1960s as a way to describe how new agricultural innovations spread, Diffusion of Innovations focuses solely on constructs related to dissemination and addresses both individual and organizational levels [28]. Specifically, it provides a framework for understanding how an innovation (i.e., "an idea practice or object that is perceived as new by an individual or other unit of adoption" [32]) is diffused, or "communicated through certain channels over among the members of a social system" [32]. Diffusion of Innovation's constructs are broad, which allow nutrition researchers and practitioners more flexibility when applying the framework [28]. This framework consists of four overarching elements: *attributes of the innovation*, *communication*, *time*, and *social systems*. *Attributes of the innovation* are its perceived characteristics relating to relative advantage (i.e., how much better is the innovation to what it would replace); compatibility (i.e., how consistent the innovation is with experiences and needs of the potential adopters); complexity (i.e., the perception of how difficult the intervention is to understand and use); trialability (i.e., the "degree to which the innovation may be experimented with on a limited basis" [32] before deciding to fully commit to the innovation); and observability (i.e., how visible the results are to others). The *communication* element is concerned with the communication channels, particularly whether the traits of the individuals or organizations communicating are homophilic (similar) or heterophilic (different). The element of *time* addresses the decision-adoption process and rate of adoption. This element provides a way to describe the different groups of adopters. Finally, the *social system* is concerned with how the different members and structures within the system work to influence the innovation adoption. Within the field of nutrition, Diffusion of Innovations has been used to describe the dissemination and/or the use of nutrition education curricula in New York state's secondary schools [37] and Vermont elementary schools [38].

TABLE 6.3 Descriptions of Example D&I Frameworks

Framework	Year Developed	Field of Origin	D&I Focus[a]	Key Constructs
Diffusion of Innovations [32]	1960s	Rural sociology	D	Attributes of the innovation
				Communication
				Time
				Social systems
RE-AIM [33]	1999	Health promotion	D = I	Reach
				Effectiveness
				Adoption
				Implementation
				Maintenance
PARIHS [34]	2004	Nursing	I	Evidence
				Context
				Facilitation
PRISM [35]	2008	Health care	I > D	Perspectives of the intervention
				Characteristics of the recipients
				Implementation and sustainability infrastructure
				External environment
ISF [36]	2008	Violence prevention	D = I	Prevention synthesis and translation system
				Prevention support system
				Prevention delivery system
CFIR [30]	2009	Health promotion	I	Intervention characteristics
				Inner setting
				Outer setting
				Characteristics of individuals
				Process of implementation

[a]*Focus on D&I spectrum based on Tabak and colleagues' rating [28].*
D, dissemination only; *D = I*, equal focus on D&I; *I > D*, greater focus on implementation; *I*, implementation only.

B RE-AIM Framework

The RE-AIM framework, developed by Glasgow and colleagues [33], was specifically designed to provide a structure to develop, conduct, and report on studies of health innovations that address both internal (related to efficacy) and external (related to generalizability) validities. As such, RE-AIM seeks to move beyond the pervading efficacy-based research evaluation paradigm toward a research paradigm that provides better evidence to assess the population-level impact of health-related innovations in real-world settings. Equally focusing on D&I, RE-AIM can be used at the individual, community, and organizational levels of the socioecological model [28]. RE-AIM gets its name from its five well-defined and readily operationalizable constructs:

- *Reach*: the proportion of the target population that participates in the assessment of the innovation and how that population reflects the local population;
- *Effectiveness*: ability of the innovation to change behaviors, mediators, and anthropometrics in the desired direction;
- *Adoption*: where and who take on the program;
- *Implementation*: how the intervention is delivered, including proportion of activities completed, fidelity, and cost;
- *Maintenance*: extent to which the program and its effects are maintained over time.

The RE-AIM framework is widely used in nutrition and other health fields. A total of 370 articles that have used the framework have been identified, including 8 nutrition interventions and 58 physical activity interventions [39]. Nutrition interventions that have used RE-AIM to guide their evaluation and reporting include Huye and colleagues' nutrition intervention for adults in the lower Mississippi Delta [40] and Toobert and colleagues' clinical trial exploring the impacts of the Mediterranean Lifestyle Program on Type 2 Diabetes [41].

C Promoting Action on Research Implementation in Health Services

Developed by researchers and practitioners at the Royal College of Nursing to provide a framework to support the delivery of evidence-based nursing practices, promoting action on research implementation in health services (PARIHS) posits that the dynamic relationships among *evidence*, *context*, and *facilitation* drive the successful implementation of effective, evidence-based practices [34]. When all these factors are "high," implementation success is theorized to be more likely. *Evidence* involves data from multiple sources, including effectiveness research, clinical experience, and patient satisfaction. Evidence is high when the data from the various sources are aligned (i.e., effective program, positive clinical experiences, and high patient satisfaction). *Context*—described as the environment in which the innovation will be received or implemented—impacts whether or not the environment is supportive of the innovation and includes three themes: culture, leadership, and evaluation. *Facilitation* is the process that makes implementing the innovation easier and involves a facilitator with a purpose, role, and skills/attributes. PARIHS can be used at the individual, organizational, and community levels of the socioecological model [28]. Relevant to nutrition research, Perry and colleagues [42] applied PARIHS in their qualitative examination of factors needed to improve nutrition and hydration practices within a residential care center.

D Practical, Robust Implementation, and Sustainability Model

Practical, Robust Implementation, and Sustainability Model (PRISM), which has a larger emphasis on implementation than dissemination [28], was developed to provide both a practical and robust conceptual framework to improve the translation of innovations within health-care settings into practice [35]. This framework builds from four existing and well-tested frameworks, including Diffusion of Innovations [32] and RE-AIM [33]. This model posits that *perspectives of the intervention* interact with the *characteristics of the recipients* at both the organizational and patient levels. This interaction is then influenced by the *implementation and sustainability infrastructure* and *external environment*. Taken together these four domains (and their subelements) impact outcomes related to adoption, implementation, and maintenance at organizational level, and reach and effectiveness at the patient-level. The model proposes these domains as "leverage points" that can be acted on to support or improve implementation. PRISM is often used within health-care settings, and an example of its application relevant to nutrition is Liles and colleagues' [43] qualitative exploration of the facilitators and barriers to the implementation of a colorectal screening outreach program within a large health-care organization.

E Interactive Systems Framework

With roots in the violence prevention field, Interactive Systems Framework (ISF) was developed to address the "how to" gap that prevents effective innovations tested in research trials from being well-implemented in real-world settings and having a greater population health impact [36,44]. This framework—which equally focuses on D&I and can be applied to the individual, organization, community, and systems levels of the socioecological model [28]—presents an approach to address key challenges that prevent evidence-based programs from being implemented by building capacity at two levels: specific (e.g., directly related to the delivery of the program) and general (e.g., more transferable) [36]. To do this, ISF posits that three systems must act together to allow an evidence-based program to be successfully disseminated and implemented into a real-world setting. The *Prevention Synthesis and Translation System* synthesizes and translates information about innovations and distills it into understandable and actionable information that can be used by (potential) delivery agents. The *Prevention Support System* builds specific and general capacity (i.e., the ability, skills, and motivations to conduct and sustain prevention work at the individual, organization, and systems level) to conduct the prevention activities. The *Prevention Delivery System* facilitates the actual delivery activities necessary to implement the innovation (e.g., recruiting participants, conducting education sessions). ISF rejects a top-down approach and requires stakeholders across levels (e.g., researchers, managers, delivery agents) to be involved in each of the three systems. Leading D&I scholars view the ISF framework as integrating many of the concepts found across other D&I frameworks [45]. While ISF has been used to support and/or explore the D&I of health innovations, there are no known published studies using the framework in the context of nutrition (as of mid-2016). However, there are a few relevant

studies that are currently ongoing, such as Zoellner and colleagues' use of ISF to support the D&I of an effective intervention to reduce sugar-sweetened beverage intake into Appalachian communities through the Virginia Department of Health [46].

F The Consolidated Framework for Dissemination Research

Developed by US Veteran Health Administration researchers [30], Consolidated Framework for Implementation Research (CFIR) is a meta-theoretical framework that brings together constructs from the 19 established implementation frameworks. CFIR does not depict relationships between constructs; it provides an overarching typography that allows researchers and practitioners to identify relevant constructs. CFIR—which addresses only implementation and provides constructs that can be readily operationalized at the organizational and community levels of the socioecological model [28]—consists of 37 constructs that are organized into five domains. Constructs within the first domain, *intervention characteristics*, address the core and peripheral attributes of the innovation, including intervention source, complexity, design quality and packaging, and cost. Constructs relevant to the context that surrounds the innovation's implementation are found in two domains: *inner setting* (i.e., the immediate organization context) and *outer setting* (i.e., the external economic, political, and cultural contexts). The line between these domains is dynamic and may not always be clear. The domain *characteristics of individuals* houses five constructs related to traits of the agents within the implementation process. The fifth domain focuses on constructs that relate to the *processes* of implementation, including planning, engagement, execution, and evaluation; processes "may be formally planned or spontaneous, conscious or subconscious; linear or non-linear" [30]. While CFIR is relatively new, it has been used in 359 studies looking at health innovations [47]. Related to nutrition, CFIR has been applied in research to define next steps for nutrition research at the molecular to population levels [48]; to develop a web-based clinical decision-support system for dental hygienists that would allow for chairside primary care screening, including screening for dietary risk factors for dental caries risk [49]; and to understand stakeholder experiences implementing a weight management program for veterans [50].

Identifying a D&I framework, along with the identification of the appropriate trial type for the stage of translational research, forms the foundation for the conduct of translational research. While these six D&I frameworks are not an exhaustive reflection of those available [28], they provide indication of construct overlap and differences across frameworks. Consideration of the trial type and framework will guide the selection of D&I research methodology, including the study design.

IV COMMON STUDY DESIGNS AND APPROACHES IN D&I RESEARCH

Previous chapters in this book detail important concepts and methodology related to the design and evaluation of nutrition interventions. These prior chapters primarily focus on the opportunities and challenges when evaluating efficacy and effectiveness, focus on internal validity, and highlight the value of RCTs. Indeed, the hierarchy of research evidence clearly posits RCTs as the most robust and strongest level of evidence in efficacy and effectiveness research trials [51]. However, as described in Tables 6.1 and 6.2, research questions in translational research often move beyond by only evaluating effectiveness. Therefore, an RCT design may not always be the most appropriate design, nor the design that can provide the best evidence to address the important questions posed by organizational- and systems-level stakeholders [52,53]. Likewise, D&I research addressing complex public health problems may not be suited to RCT, due to less-controlled settings and demand for pragmatic approaches [54]. This challenge is evidenced in the D&I field, as the emerging frameworks demand new perspectives for study designs that are both appropriate and rigorous to address D&I research questions. As such, several D&I scholars have challenged the notion that RCTs are the gold standard of D&I research and have encouraged broader use and acceptance of novel randomization techniques, quasi-experimental studies, and natural experiments. Table 6.4 illustrates several research designs that are valued in the D&I field and have also been shown to provide compelling evidence in the translational research process.

Stepped-wedge design and randomized encouragement trials are two examples of novel randomized designs that offer alternatives to the traditional parallel RCT [55,56]. In both of these designs, randomization can occur at the individual level, but is often used when individual randomization is not desired or possible. As such, randomization typically occurs at the group or setting level (i.e., cluster). In the stepped-wedge design, an intervention is rolled-out sequentially to the participants (either as individuals or clusters) over several time periods or "steps," and the order in which they receive the intervention is random [55]. In this design, more clusters receive the intervention toward the end of the study, relative to the earlier stages. By the end of the study all individuals will

TABLE 6.4 Examples of Research Study Designs for the D&I Phases of Translational Research

Study Design	Design Features
Randomized Designs	
Stepped-wedged design [55,56]	The intervention is rolled-out sequentially to the participants over several time periods. Individuals are randomized to the order in which they receive the intervention. By the end of the study, all individuals will have received the intervention. Data collection points are incorporated each time period where a new group (step) gets the intervention. Intervention effects are assessed by comparing data points of participants in the control section of the wedge with participants in the intervention section. This design can also be applied to clusters of individuals.
Randomized encouragement trial [56]	This design encourages study participants randomized to the intervention condition to participate in the intervention or to choose among specifically defined intervention components from a menu of options. Control participants are not offered the intervention nor encouraged to participate. This design can include randomization at individual level or higher, and is often used to mimic the delivery of preventive services in real-world settings.
Quasi-Experimental Designs or Nonrandomized Design With or Without Controls	
Pre–post [11,56]	Measures variables at a single point before the intervention begins and at a single point after the intervention. In this design, there may or may not be a control or comparison group.
Interrupted time series [11,56,57]	Consecutive observations are measured and interrupted at a specific point in which the intervention, service, or policy will occur. These consecutive observations are made before, during, and after implementation of the intervention. Effects are assessed by examining fluctuations in the slope or level of the series following the intervention implementation.
Multiple baseline [56]	This design is a form of an interrupted time series design. Numerous intervention components can be initiated at the onset of the study, and then components are selectively removed to understand which ones are most effective. Alternatively, components can be consecutively added until the intervention has achieved the desired effect. This design provides the ability to study different components of the intervention, in contrast to designs that only allow evaluation of a whole intervention package.
Regression discontinuity design [12,56]	Rather than random assignment, this design involves assigning individuals to the intervention and comparison conditions based on cut-point scores for the targeted variable. This design can be applied to groups, and can also include two or more intervention conditions. Intervention effects are assessed by studying the regression line, and examining if the intervention groups' regression line is discontinuous from the comparison group.
Natural Experiments [52,53]	
	Natural experiments typically involve situations where an intervention cannot be manipulated by the researcher. This can involve the study of existing or newly developing programs or policies in naturally occurring situations. Nonexperimental and quasi-experimental designs can both be used to study natural experiments.

Source: Reprinted from J. Zoellner, L. VanHorn, P. Gleason, C.J. Boushey, What is translational research? Concepts and application in nutrition and dietetics, J. Acad. Nutr. Diet., 115 (7) (2015) 1057–1071, with permission from Elsevier.

have been exposed to the intervention. Data collection points are incorporated in each time period where a new group or "step" gets the intervention. Reviews on stepped-wedge designs illustrate the breadth of these studies across diverse disease conditions, health service delivery, social policy, and criminal justice areas [55,58,59]. Two examples of a stepped-wedge randomized-controlled design include a multiple behavior healthy lifestyle intervention for clients attending a residential substance abuse treatment [60] and an intervention to improve cardiovascular disease prevention and care for high-risk patients in primary care settings [61]. While randomized encouragement trials are distinct from stepped-wedge design, they are also often used to mimic the delivery of preventive services in real-world settings [56].

A quasi-experimental study lacks random assignment. With respect to internal validity, quasi-experimental studies are often viewed as inferior to randomized experiments. However, quasi-experimental designs have obvious value when conducting research in the context of real-world practice- and community-based settings, where external validity is equally valued. While not an exhaustive list, features of four quasi-experimental designs including pre–post [11,56], interrupted time series [11,56,57], multiple baseline [56], and regression discontinuity [12,56] designs are illustrated in Table 6.4.

To further illustrate the interrupted time series, consecutive observations are measured and interrupted at a specific point in which the intervention, service, or policy will occur. These observations are made before, during, and after implementation and effects are assessed by examining fluctuations in the slope or level of the series following implementation. A few examples of interrupted time series designs include nutrition labeling provided at the point of selection [62] and pay for performance incentives on the quality of primary care and outcomes among hypertensive patients [63].

Additionally, the value of natural experiment cannot be discounted when conducting D&I research [52,53]. Natural experiments involve situations where exposure to the experimental and control condition are determined by factors outside the control of the researchers. When examining existing or newly developing programs or policies in naturally occurring situations, natural experiments can be of immense value and can include both nonexperimental and quasi-experimental designs. A recent systematic review of natural experiments in the context of obesity-related outcomes highlight the strengths and limitations of policy and built environmental changes on body mass index, diet, and physical activity outcomes [64]. The demand and value of rigorously evaluated natural experiments are increasingly being recognized among policy makers, D&I scholars, and funding agencies.

In addition to considering the study designs illustrated in Table 6.4, the use of participatory research approaches are often necessitated when conducting D&I research [65–67]. Given that stakeholder involvement is at the core of translational research efforts [68], participatory research tactics are needed to engage and promote trusted collaboration among diverse stakeholders, health-care professionals, and multidisciplinary academic researchers. Community-based participatory research (CBPR) [65], practice-based research networks (PBRNs) [66], and systems science methods [67] are all examples of participatory research approaches. CBPR is an important approach to develop and execute health interventions among marginalized populations, and a key strategy to translate research into practice to help reduce health disparities [69–71]. Effective CBPR initiatives use the collective knowledge, expertise, and resources gained through community-academic partnerships to develop and execute culturally-effective interventions, as prioritized by the community [72–74]. The CBPR approach is designed to ensure community participation in all aspects of the research process and build equitable community-academic partnerships [69]. Gaining trust in vulnerable communities and promoting intervention program sustainability have been noted as essential elements of CBPR [65]. PBRN refer to the collaboration of a group of clinicians and/or practices to address practice- and community-based health-care questions. A primary goal of PBRNs is to provide capacity building activities and to engage clinicians in the translation of evidence-based findings into primary care practices. These PBRNs can be at the regional, state, local, or national levels. Examples of collaborating institutions can include, but are not limited to, academic medical centers, individual group practices, Federally Qualified Health Center, local or regional hospitals, and community health centers [66]. Systems science methods take into account the complex and dynamic nature of public health relevant to D&I research because interactions are occurring among multiple levels within a system and across systems [67]. One description of systems science within health-care or public health settings describes vertical and horizontal components that may interact to translate an evidence-based intervention into practice [75]. Vertical components of a systems-based approach can be operationalized from a municipal, regional, state, and federal level, and can also include the involvement of both the staff and organizational decision-makers in the development, implementation, and testing processes. Horizontal components of a systems-based approach include the engagement of several sectors within a local region that can aid in the implementation and maintenance of evidence-based interventions [75]. Systems science approaches typically include social network analysis, agent-based modeling, and systems dynamics [3,67].

Similar to the use of participatory research approaches, mixed-methods research designs are common in D&I research [76,77]. Mixed-methods research designs combine the strengths of both quantitative and qualitative methods in a single inquiry, to gain a more complete perspective [76,77]. While a complete description of mixed-methods research designs are beyond the scope of this chapter, there are a variety of mixed-methods research designs (e.g., convergent parallel, explanatory sequential, exploratory sequential, embedded, transformative, multiphase) that have relevance to answering research questions that examine the translation of evidence-based practices in real-world clinical and community settings. Conducting complex and multilevel D&I interventions and evaluations requires close considerations for the trade-offs in study designs and must be considered concurrently when selecting appropriate D&I measures.

V MEASURES IN D&I

As D&I research simultaneously assesses different types of outcomes that address aspects of the D&I process and of the innovation's effectiveness [78], there is a diversity of quantitative and qualitative measures to assess D&I outcomes. Related to the D&I process, dissemination (e.g., reach) [78] and implementation

(e.g., fidelity, acceptability, penetration, cost) [78,79] outcomes are assessed as well as characteristics of the service system through which the innovation is being disseminated and implemented (e.g., organizational capacity, organizational behavior, policy adoption) [80]. Table 6.5 highlights how constructs within different D&I-focused outcomes have been measured. Related to intervention effectiveness, D&I outcomes relate to an individual (e.g., behavior change, anthropometrics) [79] and/or population (e.g., population-wide consumption patterns, disease incidence) health. Additionally, D&I researchers are often interested in interrelationships between D&I and effectiveness outcomes (e.g., *how does implementation impact individual outcomes?* and *what is the cost-effectiveness of the intervention?*) [78]. To measure these outcomes, D&I researchers use quantitative and/or qualitative approaches.

Quantitative measures produce numerical data about the outcome or framework construct in question. Records of material, personnel, and travel expenses may be kept to determine implementation costs. To assess reach, tallies of the numbers and characteristics of potential participants approached may be kept and compared to those joined the intervention. Checklists, including items such as proportion of material delivered, concepts covered, and rating of delivery, are often used to measure implementation fidelity. Surveys and questionnaires can be used to assess well-defined constructs, such as organizational climate.

Within D&I research, qualitative measures are used both to assess outcomes and to inform the development or

TABLE 6.5 Example Measures by General Constructs Across D&I Outcome Levels

Outcome	Sample Construct Measured	Construct Description	Types of Measures	Example of Construct Assessment in Literature
Dissemination	Reach	Total number, proportion, and representativeness of individuals who participate in a given initiative	Tracking forms	Determining the reach of a multilevel community intervention with a media component to reduce childhood obesity [81]
Implementation	Fidelity	Integrity of how the initiative was delivered, e.g., delivered as intended, adherence to protocols	Checklists, observations	Using a fidelity checklist to completion of a nutrition education curriculum delivered by teachers [82,83]
	Acceptability	Perceptions of participants that the initiative met their needs and/or perceptions of organizational stakeholders that the initiative fits within their system	Surveys, interviews	Determining participant satisfaction with a community-based intervention to reduce sugary beverage intake [84]
	Penetration	Who participates and how often, at the participant and/or provider levels	Tracking forms, surveys, interviews	Assessing the uptake of standard terminology and definitions for texture modified foods and fluids [85]
	Cost	Financial resources needed to deliver the intervention, including recruitment, implementation, and maintenance and how those costs are reflected in changes in other outcomes, such as health	Tracking forms	Determining the cost-effectiveness of a physical activity intervention for older adults [86]
Service system	Organizational capacity	Existing knowledge, skills, and infrastructure within an organization that gives it the ability to deliver services	Surveys, observations, focus groups, interviews	Assessing the organizational capacity of public health organizations to deliver chronic disease prevention programs [87]
	Organizational behavior	How an organization responds to a specific initiative	Record reviews, surveys, observations, interviews	Tracking changes in school cafeteria food purchasing following an intervention [88]

refinement (e.g., cultural adaptations) of D&I strategies. Qualitative measures are able to explore aspects of D&I constructs that may not be directly captured through quantitative measures. Additionally, qualitative measures can elucidate information that might not be readily known or previously considered by a researcher (e.g., specific organizational norms). Focus group and interview protocols, observation notes, and analysis of process artifacts (e.g., meeting minutes, brainstorming sheets) are commonly used qualitative measures. As one example, the CFIR guide provides a list of possible interview or focus group questions for all constructs within the framework as well as templates for observations and meeting notes [47]. Often, qualitative measures are used in tandem with quantitative measures to provide more robust data [76,77].

Unfortunately, the progress of D&I research is being somewhat slowed by conceptual and practical barriers to measuring outcomes that extend beyond the innate complexity of the field [89]. Martinez and colleagues have identified six instrumentation issues: (1) growing number of frameworks and lack of standardization of constructs among frameworks; (2) lack of reporting of measuring of psychometric properties; (3) use of home-grown and adapted instruments; (4) not choosing the most appropriate evaluation method and framework; (5) lack of pragmatic measures; and (6) few decision-making tools [89]. These issues have arisen due to some of the characteristics of D&I research. First, D&I research is relatively new and is spread across specific content fields (e.g., violence prevention, nursing, health care). As researchers within and across fields arrive at solutions to address gaps in knowledge, this knowledge is not always efficiently shared within and across fields. Next, there is often a need for measures, such as fidelity checklists and satisfaction surveys, to reflect specific content of a D&I project. Lastly, existing measures may need to be practically adapted to meet the needs of the target population (e.g., translation for non-English speaking populations) and the needs of the organization providing the intervention (e.g., adapting measures to be conducted over the phone or via the Internet instead of in-person when the population to be served is highly spread out, such as in a rural area) [14].

The measurement challenges, mentioned previously, should not deter interested nutrition researchers and practitioners from engaging in translational and D&I research. Rather, they provide opportunity for interested stakeholders to thoughtfully approach the selection and measurement of D&I outcomes. Notably, the field is undertaking many of the recommendations laid out by Martinez and colleagues [89] as ways to address these measurement issues and make them available to others researchers and stakeholders. For example, in efforts to harmonize framework constructs, D&I scholars are cross referencing theory constructs related to diffusion [90], adoption [80,91], and implementation [30,31]. Additionally, efforts are ongoing to consolidate the measures. A working group of 23 leading D&I researchers, practitioners, and decision-makers identified at least 17 (13 of which are from the health field) living repositories (e.g., Society for Implementation Research Collaboration (SIRC) instrument review project [92]) and static reviews (e.g., systematic reviews published in scholarly journals [93,94]) of constructs from frameworks and relevant measures [95]. Finally, and of particular importance, D&I scholars from SIRC were awarded a 3-year NIH grant (2015–18) to establish a battery of reliable, valid, and pragmatic measures that can be used to advance D&I research [92,96].

D&I research inherently straddles a line between scientific rigor and pragmatism [14]. When done well, D&I research appropriately balances internal and external validity factors and produces outcomes that increase the opportunity to efficiently translate scientific discoveries to improve population health. The concepts presented in this chapter have provided an overview of concepts, challenges, and opportunities to be considered when conducting D&I projects and research. The remainder of this chapter presents two translational research studies of the DPP and shares resources to allow nutrition researchers and practitioners to further expand their knowledge and skills about D&I and translational research.

VI EXAMPLES FROM THE LITERATURE

To further illustrate several translational research concepts presented in this chapter, examples from the literature focused on the DPP are presented. The DPP is an effective lifestyle intervention designed to reduce diabetes incidence among prediabetic adults. Between 1996 and 2001, 3234 nondiabetic persons with elevated fasting blood glucose were randomized into one of three arms of an RCT: (1) lifestyle modification program which focused on diet, physical activity, and weight loss/maintenance (DPP); (2) metformin medication; or (3) placebo. In brief, the study showed that the incidence of type 2 diabetes could be reduced by 58% through participation in the lifestyle modification program (DPP) [97]. Importantly, the positive effects achieved by the diabetes lifestyle intervention exceeded the effects achieved by metformin medication. Findings were so strong that the blinded treatment phase was stopped a year early. Based on these results, there have been numerous efforts to disseminate the DPP into routine practice to improve individual- and population-level health. The DPP has been adapted and translated into a variety of populations (e.g., gender, race) and real-world, practice-based settings (e.g., out-patient

clinics, primary care, YMCAs, churches, work places, online platforms) both in the United States and abroad [98–101]. Likewise, these efforts have utilized an assortment of translational strategies, cultural adaptations, frameworks, study designs, and evaluation of individual- and organization-level outcomes [98–101]. Translation of the DPP in two different settings, including a hybrid effectiveness–implementation type 3 trial and a quasi-experimental comparative effectiveness trial, are further critiqued here.

The first example is a clinical demonstration project of the DPP being conducted by Damschroder and colleagues [22]. In this hybrid effectiveness–implementation type 3 trial, the primary aim is focused on the implementation of the DPP in three VA medical centers. Secondary aims are focused on participant effectiveness outcomes including weight and hemoglobin A1c. Additional aims include cost-effectiveness and budget impacts from the VA health systems' perspective. For the primary aim, development and evaluation of the implementation strategy is being guided by Simpson and colleagues' theoretical model for translating research into practice [102]. Implementation phases pertain to training, leadership decision, exploratory use, and routine use. This is a nonrandomized trial, as neither the VA sites nor VA staff delivery agent will be randomized. However, the goal is to systematically assign about 720 VA participants into the DPP program or the established VA Move! Program [103]. This pragmatic trial is guided by integrating both the RE-AIM [33] and CFIR [30] frameworks. The study protocol includes specific quantitative and qualitative research questions across each of the RE-AIM dimension. A few key sample research questions include: (1) What are the characteristics of eligible patients that enroll (in VA DPP and VA MOVE!) as compared to eligible patients who don't enroll (reach); (2) What are the changes in weight and A1c at 6 months (effectiveness) and 12 months (individual-level maintenance); (3) How many sites agree to implement the VA DPP (adoption); (4) To what extent are the VA DPP and VA MOVE! delivered as intended; and (5) What are the prospects of continuing or restarting a VA DPP (organizational-level maintenance). The CFIR framework will be used to thoroughly understand the barriers and facilitators that influence adoption and implementation of the DPP, assessing constructs across the five major CFIR domains including intervention characteristics, outer and inner setting, characteristics of individuals, and implementation process. Mixed method sources of data include staff interviews, fidelity assessments, cost tracking, and patient-level measures including anthropometrics, clinical outcomes, and patient surveys (e.g., quality of life, interviews). This trial is an exemplary representation of a rigorous, mixed-method, D&I research study. By using sound D&I frameworks, a type 3 hybrid trial design, and carefully selected mixed-methods measures, findings from this research will answer important practice-based questions pertaining to implementation, effectiveness, and cost-effectiveness [22].

The second example is a quasi-experimental, multisite study that examines four 12-week comprehensive lifestyle behavior-change programs adapted from the DPP [104]. In this comparative effectiveness trial, the four groups include face-to-face, DVD, Internet modalities, as well as a self-selection group that choses their own intervention modality. The primary aim of this study was to determine the comparative effectiveness of these modalities in influencing individual-level diabetes risk factors including weight and clinical cardiovascular risk factors (e.g., blood pressure, glucose, lipids, waist circumference) at 3 and 6 months. Neither participants nor communities were randomized. Rather communities were allocated to one of the four intervention conditions based on community characteristics and the community's capacity to deliver the intervention. For example, communities that did not have adequate high-speed Internet infrastructure where not allocated to the intervention group. The research team trained and certified community sites' registered nurses, dietitians, and lay health coaches on the study protocol including screening and measurement techniques as well as content and support for the interventions. In total, 555 overweight participants from 26 intervention sites and 8 rural underserved communities were assigned to an intervention group. At 3 and 6 months, all intervention groups had significant decreases in body weight and significant improvements in the proportion of participants with cardiovascular risk factors. However, statistical difference among groups was noted at both 3 and 6 months. Compared to other groups, participants in the self-selection group experienced the largest average weight loss at 3 months, and participants in the face-to-face group experienced the largest weight loss at 6 months. This study illustrates the pragmatic decisions demanded when conducting CER in real-world and underserved community settings. However, there is no D&I framework identified in this study and no reported dissemination, implementation, or qualitative outcomes. The external validity would be enhanced by assessing and reporting barriers and facilitators that influenced adoption, implementation, and site- and/or community-level maintenance of the interventions. As one example, evaluation of intervention fidelity among the community sites and staff would inform the potential to translate the programs into other communities.

Limited focus on D&I outcomes is not isolated to the aforementioned comparative effectiveness trial of four DPP intervention modalities [104]. In a recent systematic review of 44 studies conducted to characterize the literature on the DPP translation from 2004 to 2013, 15 studies

described cultural adaptations and 38 studies reported on implementation [98]. Of these 38 studies that reported on implementation, only 11 (28%) described the use of an implementation framework. The most common frameworks include the following: CBPR (5 studies), Diffusion of Innovation (3 studies), and RE-AIM (2 studies). At the organizational level, the most common type of implementation outcome assessed was feasibility ($n = 32$ studies; 84%), such as cost, staffing, and space. Other types of organizational-level outcomes and the proportion of studies that were reported included appropriateness (53%), sustainability (39%), acceptability (37%), adoption (37%), fidelity (34%), and penetration (24%). Findings from this review also highlight shortcomings with insufficient detail provided on the measurement and evaluation of these organizational-level outcomes [98].

While substantial effort has been invested in translating the DPP into numerous real-world, practice-based settings and for diverse populations, concerted effort is still needed to elevate the focus on D&I frameworks, methods, and measures. The translational research on evidence-based practices into real-world clinical and community settings demands heightened attention and prioritization on organizational- and systems-level outcomes. The additional resources provided later are intended to assist nutrition researchers and practitioners in obtaining additional information on advanced D&I topics and staying abreast of the rapidly expanding field of translational research.

VII ADDITIONAL RESOURCES

A Leading D&I Centers and Agencies

In the past several years, there have been notable investments to speed up the translation of scientific discovery into patient and community benefit more quickly. One key example is efforts led by the NIH and the establishment of the *National Center for Advancing Translational Science (NCATS)* in 2012 [105]. The mission of the NCATS is "to transform the translation of scientific discoveries so that new treatments and cures for disease can be delivered to patients faster." The NCATS is responsible for administering the *Clinical and Translational Science Award program*. This program supports a national network of over 50 medical research centers that are collaborating to develop and disseminate innovative methods and technologies that improve the efficiencies of turning scientific discoveries in clinical advances. Another example is the *Implementation Science team lead by the Division of Cancer Control and Population Sciences within the National Cancer Institute* [106]. This team is addressing the degree to which health interventions can fit within real-world public health and clinical services systems. Additional illustrations are two federal groups that have emerged as leaders in the area of CER, including the *Agency for Healthcare Research and Quality* and the *Patient-Centered Outcomes Research Institute*. While these examples are clearly not an exhaustive list, they provide an indication of investments into accelerating the translational research process and provide a hub of supplementary resources to this chapter. Many of these leading centers and agencies also offer advance training and seminars in translational and D&I science, host annual research conferences, and have free Listserv subscriptions.

B Research Tools for Identifying and Reporting Diverse Trial Types

Two additional resources for understanding the conduct and reporting of diverse trial types include extensions of the *Consolidated Standards of Reporting Trials* (CONSORT) statement and the *Pragmatic-Explanatory Continuum Indicator Summary* (PRECIS). The main CONSORT statement is a 22-item checklist to improve the reporting of an RCT, based on the "standard" two-group parallel design [107]. To improve the reporting of my diverse trial types, including many of those used in D&I research, several modifications are now available including extensions for cluster trials [108], noninferiority and equivalence trials [109], and pragmatic trials [110]. For example, the PCT extension adds specific guidance of good reporting for 8 of the 22 checklist items, including background, participants, interventions, outcomes, sample size, blinding, participant flow, and generalizability [110]. The PRECIS provides 10 domains to identify the extent to which a trial is pragmatic [111]. The PRECIS domains illustrate aspects such as eligibility criteria for trial participants, flexibility with experimental intervention, flexibility of comparison intervention, intensity of follow-up of trial participants, intensity of measuring participants' compliance with the prescribed intervention, degree of practitioner expertise, intensity of measuring practitioners' adherence to the study protocol, and the nature and scope of the trial's primary outcome. The intent of the PRECIS tool is to assist researchers and practitioners in designing and characterizing trials and to help highlight the multidimensional nature of the pragmatic-explanatory continuum [111]. The PRECIS criteria have been applied in several studies relevant to nutrition outcomes including weight loss trials in obese patients with comorbid conditions [112] and eHealth cancer prevention and control interventions [113].

C Resources for Selecting D&I Frameworks and Measures

Rabin and colleagues' glossary of D&I terminology provides researchers and practitioners seeking to conduct

translational resource within nutrition and other health fields to navigate the often conflicting terminology needed to identify the focus of a D&I study and to select the proper framework. This resource was first published in the *Journal of Public Health Management and Practice* [114] and updated in 2012 for the *Dissemination and Implementation Research in Health: Translation Science to Practice* textbook [29].

Regarding the selection of frameworks, Tabak and colleagues' 2012 review of D&I frameworks published as an open access article in the *American Journal of Preventive Medicine* provides a synthesis of 61 D&I frameworks [28]. To aid researchers and other stakeholders in selecting the framework that best fits their needs, frameworks are organized by D&I focus as well as construct flexibility. Levels of the socioecological framework are also presented. Additionally, some of the research teams host their own websites that provide greater detail about constructs and highlight articles that have used the frameworks. Two notable website examples are the *CFIR technical assistance* [47] and the *RE-AIM working group* [39].

There are also a number of measurement resources. Notably, Rabin and colleagues present a compilation of 17 such resources identified by a working group of leading D&I scholars, practitioners, and decision-makers [95]. Two of the publicly available resources are (1) the *CFIR technical assistance website*, which includes relevant quantitative and qualitative measures [47] and (2) the National Cancer Institute's *Grid-Enabled Measures (GEM)* portal, which allows D&I stakeholders to add evidence about relevant constructs and measures and share data sets [115,116]. As of mid-2012, there were 1202 measures assessing 357 constructs compiled in GEM. Additionally, there is the *SIRC* website which hosts a repository of 450 measurement instruments that have been collected through the ongoing Instrument Review Project. Measures are organized by CFIR constructs and have been rated based on their empirical validation [92]. This resource is available to SIRC members.

D Books and Journals

The *Dissemination and Implementation Research in Health: Translation Science to Practice* textbook is one of the most comprehensive textbook on the theory, frameworks, methodology, and analysis of setting- and population-specific D&I research [3]. Another relevant textbook is *Improving Patient Care: The Implementation of Change in Health Care* [117]. Finally, while D&I research is featured across numerous scientific journals, a few journals dedicated to this research paradigm include: *Implementation Science* published by BioMed Central and *Translational Behavioral Medicine* published by Springer. These books and journals are recommended for nutrition scholars wishing to advance their proficiencies in translational and D&I research.

VIII CONCLUSIONS

Since several definitions and models of translational research exist in the literature, it can hold diverse meanings across individuals and disciplines. Despite variances in terminology of translational research phases, the need to accelerate the lag time from scientific discoveries to widespread clinical and population health impact is well-recognized. This chapter focused on the D&I research concepts, processes, and factors that influence the successful integration of evidence-based interventions into real-world clinical and community settings. Fortunately, there are numerous D&I frameworks, study designs, and measures to select from. Exploring how D&I concepts have been used in other health and nutrition-relevant studies, as well as using the resources provided in this chapter, can aid nutrition researchers and practitioners in the critical evaluation and careful application of D&I methodology that best meets the needs of their projects.

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Chapter 7

Overview of Nutritional Epidemiology

Adriana Villaseñor[1], Lisa Cadmus-Bertram[2] and Ruth E. Patterson[1]
[1]University of California, San Diego, La Jolla, CA, United States, [2]University of Wisconsin-Madison, Madison, WI, United States

I INTRODUCTION

Epidemiology is the science of public health whose laboratory setting is human populations. Specifically, epidemiology is the study of the distribution and determinant of disease frequency in human populations [1]. Epidemiology addresses such questions as who gets a disease, when, and why; this information is then used to create strategies for prevention or treatment. Nutritional epidemiology is the study of how dietary factors relate to the occurrence of disease in human populations [2]. For example, observations that persons who consumed fish at least twice a week had a lower risk of incident cardiovascular disease (CVD) and stroke as well as lower risk of sudden cardiac death than persons who ate no fish [3,4] led to widespread recommendations to include fish in the diet for CVD prevention. Similarly, population-based studies demonstrating that consumption of trans-fatty acids may be associated with unfavorable blood lipid profiles have led to the decline in use of these fats by food manufacturers and outright bans by some local health departments [5–8]. Another finding by nutritional epidemiologists that has had major public health policy implications was the observation that use of multivitamins containing folic acid during pregnancy reduced the risk of neural tube defects in the fetus. Consistent evidence from numerous epidemiologic studies led to the fortification of the US food supply with folic acid as a primary prevention strategy in 1998 [9,10]. Thus, some of the major nutrition-related public health contributions of the past 50 years have been made possible by nutritional epidemiology.

One factor that distinguishes the discipline of nutritional epidemiology is the extraordinary challenge of accurately assessing exposure [11]. In epidemiology, exposure is defined as participant characteristics, lifestyle behaviors, or agents (e.g., food, medications such as hormone replacement therapy, tobacco, and sun) with which a participant comes into contact that may be related to disease risk (Table 7.1) [12]. Measuring dietary intake is particularly complex for many reasons. For purposes of illustration, we can compare the assessment challenges for two common exposures: cigarette smoking and diet. Smoking is a simple (yes/no) activity, so individuals can accurately report whether or not they smoke. Because smoking is addictive, it tends to be a consistent long-term behavior. Furthermore, because smoking is a habit, most people smoke roughly the same number of cigarettes per day (i.e., one or two packs per day). In contrast, during the course of even 1 week, an individual can consume hundreds, even thousands, of distinct food items in various combinations, making it cognitively challenging for respondents to accurately report their intake. Meals can be prepared by others (e.g., in a restaurant, by a spouse, and as prepackaged food) so that the respondent may not be cognizant of preparation details such as fat or salt used in cooking or portion size. Food choices typically vary with seasons and other life activities (e.g., weekends, holidays, and vacations). In fact, the day-to-day variability in food intake can be so large that it is not possible to identify any underlying consistent dietary pattern. In addition, foods are often a surrogate for the exposure of interest (e.g., dietary fat or fiber), which means that investigators must rely on food composition databases to calculate the exposure variable. Given the problems inherent in assessing dietary intake, it is not surprising that despite some of the important contributions noted previously, it has been difficult to obtain consistent and strong evidence regarding how diet affects disease risk.

The vast majority of nutritional epidemiologic research in the past 25 years has focused on the identification of foods and/or specific nutrients in foods that prevent or promote the occurrence of chronic diseases, such

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TABLE 7.1 Examples of Exposures Relevant to Nutritional Epidemiologic Studies

Exposure	Diet-Related Example	Other Example
Agent that may cause or protect from disease	Vegetable consumption may be protective for colon cancer	Physical activity may be protective for colon cancer
Constitutional host factors	Genetic predisposition to nutrition-related disease	Older adults are more predisposed to chronic disease
Other host factors	Food preferences that determine food choices	More educated adults may have better disease screening
Agents that may confound the association between another agent and disease	Correlation between dietary constituents (e.g., a diet high in fruits and vegetables is usually low in fat)	Smokers are less likely to engage in physical activity
Agents that may modify effects of other agents	Fruits and vegetables may protect against lung cancer among smokers	Alcohol results in increased risk of lung cancer from smoking
Agents that may determine outcome of disease	Malnutrition	Medical treatment

as cancer, diabetes, and CVD. Therefore, the tools and methods of nutritional epidemiology were developed to address scientific issues unique to the biology of chronic disease, including the extensive time for disease development and the multifactorial nature of these diseases. Furthermore, epidemiologic research is generally conducted in free-living humans, which establishes associations only and precludes direct tests of cause and effect. Each of these challenges is discussed here.

A Extensive Time for Disease Development

Chronic diseases develop over years and even decades, which has important implications for the field of nutritional epidemiology. For example, the currently accepted model of colon cancer assumes that it is a multistep pathogenic pathway beginning with mutations (germ line or somatic) leading to growth of polyps (preneoplastic growths), which become adenomas and progress to carcinoma [149]. Even after an adenoma has developed, many years may elapse before it is clinically detected. Upon clinical detection of colon cancer, it is clear that a meal consumed that day, or the month before, could have no significant effect on the disease for two reasons. First, the critical period of cancer initiation and promotion likely occurred many years prior to disease diagnosis. Second, the biologically relevant exposure is the long-term or usual diet, rather than any single eating occasion. Therefore, the exposure of interest in the development of cancer occurred throughout the previous 10–20 years and perhaps the previous 5 years for CVD and diabetes.

This time lag between dietary exposure and disease occurrence presents considerable difficulties in the study of diet and chronic disease. These difficulties have been addressed in two major ways. First, dietary assessment instruments for epidemiology were developed to capture information on usual, long-term dietary intake [13]. The most common is the food frequency questionnaire (FFQ). Second, study designs developed in other areas of epidemiology were applied to studies of diet–disease risk: case–control and cohort studies. These designs were already being used to gather data on remote exposures (i.e., use of oral contraceptives and age at menarche) or life events (i.e., age at first pregnancy and number of full-term pregnancies), which proved to be useful for establishing associations of various risk factors with disease risk [1]. For example, asking detailed questions about reproductive history to women with and without breast cancer has provided useful and reliable data about breast cancer risk factors. Using the same approach, nutritional epidemiologists have asked people about retrospective diet in case–control studies or assessed current diet in cohort studies and followed participants over time to monitor incidence of disease. The difficulty with the latter approach is that it often takes years or decades for a disease to develop, and during that time, study participants may markedly alter their usual dietary habits.

B Multifactorial Nature of Chronic Diseases

Chronic diseases such as cancer and CVD have complex etiologies that are multifactorial in nature. In addition to diet, other determinants of chronic diseases include host factors (e.g., genetic susceptibility) and other lifestyle habits (e.g., cigarette smoking, physical activity, and alcohol intake). These other factors may confound our ability to find an association of dietary intake with disease risk. For example, individuals with an interest in health may eat a low-fat diet high in fruits and vegetables and have high physical activity levels. If vegetable intake is found to be associated with reduced risk of colon cancer, it can be difficult to disentangle whether it is the high vegetable intake or the low fat intake that affects disease risk. It is also possible that diet is not related to disease

risk but is merely serving as a marker for some other healthful behavior, such as physical activity or adherence to cancer screening guidelines [14].

Another illustration of the multifactorial nature of chronic disease is the complex relationship between dietary factors and a person's genetic constitution. For example, the phase II enzymes glutathione-*S*-transferases (GSTs) are upregulated by vegetables from the *Brassica* family (broccoli, Brussels sprouts, and cauliflower) such that compounds in these vegetables increase the activity of this family of enzymes [15–17]. There is also evidence that the health benefits of *Brassica* vegetables may vary depending on whether a person has any of the common genetic variations in these enzymes. Humans with GSTM1 null (approximately 50% of US Caucasians and 35% of African Americans) or GSTT1 null (approximately 15% of US Caucasians and 25% of African Americans) have greatly reduced (heterozygote) or no (homozygote) enzyme activity [16]. Among those with the null variants, compounds in the *Brassica* vegetable, such as isothiocyanates, may be metabolized and eliminated more slowly, so they may be available for a longer period of time in target tissues and organs [18]. Several studies have found stronger associations of *Brassica* vegetables with reduced risks of lung [19], breast [20], and colon cancer [21] among persons GSTM1 and/or GSTT1 null. This example of a diet–gene interaction illustrates the complexity and multifactorial nature of diet-related chronic disease development.

C Research in Human Beings

There are several important considerations with regard to conducting research in humans. Notably, population-based nutrition research or nutritional epidemiology is often conducted among healthy persons, at least at the time of initial assessment. For studies that involve dietary interventions, federal guidelines for the protection of human subjects dictate that study participants (both healthy and diseased) cannot (knowingly) be exposed to potentially dangerous dietary regimes that restrict essential nutrients or introduce known carcinogens at high levels over long periods of time. However, short-term controlled feeding studies can reasonably test the effect of potentially harmful food constituents for the purpose of learning about nutrient metabolism and disease mechanisms in relation to food intake [22]. For example, participants can be fed charred meat containing nitrosamines to learn how humans metabolize or eliminate such potential carcinogens [23,24], or they can be fed restricted diets devoid of certain nutrients to learn about nutrient absorption, metabolism, and excretion [25,26]. Indeed, such information is critical for public health recommendations regarding nutrient requirements. These tightly controlled human feeding or intervention studies are expensive and laborious and therefore are not feasible to conduct in a large-scale manner, especially when studying general population risk. For these reasons, nutritional epidemiology is primarily an observational discipline that consists largely of (1) measuring an exposure (e.g., usual dietary intake), (2) measuring an outcome (e.g., disease occurrence), and (3) using statistical techniques to quantify the magnitude of the association between these two observations and adjust for potential confounders.

II PRINCIPLES OF EXPOSURE MEASUREMENT IN NUTRITIONAL EPIDEMIOLOGY

A variety of dietary assessment methods are used in nutritional epidemiology. The choice of instrument used depends on the hypothesis being tested, the population being studied, and cost considerations. The three primary tools are FFQs, 24-hour dietary recalls, and food diaries or records. Here, we briefly describe these instruments and discuss the issues of error and bias associated with self-reported dietary intake.

A Food Frequency Questionnaire

Chronic disease risk develops over many years. As such, the biologically relevant exposure is usual or long-term diet, consumed many years prior to the appearance of clinical symptoms and disease diagnosis. Therefore, assessment instruments that only capture data on recent dietary intake (e.g., food records or recall) may not be as useful for studies examining chronic diseases that evolve over years or decades. For these reasons, FFQs have been regarded as the dietary assessment instrument best suited for most epidemiologic applications [13]. Although the design of FFQs can deviate somewhat, they typically contain the following sections: (1) adjustment questions, (2) the food checklist, and (3) summary questions.

FFQ adjustment questions permit more refined analyses of fat intake by asking about food preparation practices (e.g., removing fat from red meat) and types of added fats (e.g., use of butter vs margarine on bread). The main section consists of a food or food group checklist, with questions on usual frequency of intake and portion size. The foods are selected to capture data on (1) major sources of energy and nutrients for most people, (2) between-person variability in food intake, and (3) major hypotheses regarding diet and disease. Portion sizes are often assessed by asking respondents to mark "small," "medium," or "large" in comparison to a given medium portion size. Summary questions ask about usual intake of fruits and vegetables because the long lists of these foods

(needed to capture micronutrient intake) lead to overreporting of intake [27,28]. Since an FFQ asks about usual intake over many months, these questionnaires provide no information on dietary behaviors such as eating breakfast, snacking, or meal timing.

Note that development of an FFQ is a daunting, complex task requiring considerable understanding of exposure measurement in nutritional epidemiology, food composition knowledge, formatting and questionnaire design expertise, and computer programming resources [29–31]. A limitation of FFQs is that they may be fraught with both random measurement error and bias, which limits their usefulness [32,33]. See Section V for details regarding error in FFQs.

B Measures of Short-Term Dietary Intake: Food Recalls and Records

Food records and 24-hour dietary recalls are the other common tools used in nutritional epidemiology [13]. Food records or diaries require individuals to record all foods and beverages consumed during a specified period of time, usually 3–7 days. Participants are asked to carry the record with them and to record foods as eaten in real time. Some protocols require participants to weigh and/or measure foods before eating and review the record with a registered dietitian, whereas less stringent protocols use food models or other aids to instruct respondents on estimating serving sizes and do not engage in extensive review or documentation [34]. To obtain nutrient estimates, the food consumption information from records/diaries must be entered into a specialized software program. This data-entry step is a time-consuming task and requires trained data technicians or registered dietitians. Food records are burdensome for study participants and, due to the staff time required, are expensive to administer. Therefore, for large epidemiologic studies with tens of thousands of study participants, food records are cost prohibitive.

Some of the participant's burden can be alleviated by the use of innovative technologies such as personal digital assistants, mobile telephones, digital cameras, and other electronic devices both to record and to transmit food record data [35]. As with a paper-and-pencil food record, participants carry the electronic device with them during the assessment period. They are either asked to record each food and beverage as it is consumed or prompted every few hours to enter recently eaten foods. Electronic food records can produce intake estimates comparable to those from traditional food records or recalls [36,37]. Alternatively, mobile phones or digital cameras may be used to photograph each meal, snack, or beverage [38]. In these studies, a fiducial marker (an object of known dimensions) is often placed next to the food to provide a reference point for size. The foods are then identified and quantified either automatically using specialized software or manually by a trained technician. The participant may be asked to take both a "before" and an "after" photograph so that the amount consumed can be estimated. A third approach is the use of websites or a mobile phone application (app) which participants can use to enter their intake. These apps offer a large database of prepared and packaged foods, eliminating the need to enter caloric and macronutrient data. Users can also contribute to this database by entering packaged foods or restaurant items not already included and can also create custom meals, food combinations, or recipes to facilitate efficient entry of home-cooked dishes that they consume frequently. Advantages of app-based dietary assessment include convenience, reduced burden, and potential use in dietary change interventions [39]. Limitations include technological barriers (particularly for segments of the population where mobile phone penetrance and familiarity with app-based programs may be limited) and the potential for misinterpretation of dietary information on the part of patients and study participants [40].

Electronic approaches allow for more comprehensive recording than a paper-and-pencil survey and are particularly appealing for certain populations, such as school-aged children and adolescents [41]. Conversely, these techniques may not be suitable for individuals who are unfamiliar or uncomfortable using technology. Photograph-based methods are convenient for participants, but are limited by the lack of descriptive information about the foods and beverages in the photographs; even a highly trained dietitian can only guess at the ingredients or preparation method used. The cost of technology-based approaches compared to paper-based recalls is variable, depending on the method used for intake quantification (automated/computerized vs manual). Furthermore, the need to purchase, distribute, and recover the electronic devices limits the feasibility of this approach for large studies.

Twenty-four-hour dietary recalls are frequently used in population-based studies, such as the National Health and Nutrition Examination Survey (NHANES) [42]. Data from these recalls provide snapshots of US eating patterns and are used for formulations of dietary recommendations. A 24-hour dietary recall is a 20- to 30-minute interview in which the respondent is asked to recall all foods and beverages consumed during the previous 24 hours. Ideally, the interview is conducted with real-time direct data entry into a software program for analysis. It is important that the interviewer be well trained; tone of voice, body posture (when in-person), and reactions to participant descriptions of foods consumed can influence the quality of the data.

A single recall is considered suitable for characterizing mean intakes of groups, such as the US Department of

Agriculture (USDA) survey titled *What We Eat in America* [42]. However, for individual assessment, day-to-day variability of food intake is so high that for both records and recalls, several days of data are required to characterize usual intake for an individual. Using data on variability in intake from food records completed by 194 participants in the Nurses' Health Study [13], the number of days needed to estimate the mean intakes for individuals within 10% of "true" means would be 57 days for fat, 117 days for vitamin C, and 67 days for calcium. Unfortunately, research has shown decreases in reported energy intake, nutrient intake, and recorded number of foods with as few as 4 days of recording dietary intake [43]. These decreases in reported intake may reflect either (1) reduced accuracy and completeness of recording intake or (2) actual changes in dietary intake to reduce the burden of recording intake. Because of the participant burden, dietary recalls or records are generally only kept for 3 or 4 days. Most protocols require that one of these days be a weekend day. Although a random selection of days would be ideal, food records are generally kept for 4 days in a row to improve response rates.

In 2009, the National Cancer Institute released a web-based 24-hour dietary recall instrument, the Automated Self-Administered 24-Hour Recall (ASA24) [44–46]. The ASA24 uses a modified version of the USDA's Automated Multiple-Pass Approach, which aims to improve recall quality by giving participants several opportunities at which to recall a food item. During the first step of the recall, the participant identifies foods and beverages eaten at each meal or snack. The web-based program then asks the participant whether anything was consumed during each of the gaps between meals/snacks, offering a second chance to add forgotten items. Participants are then asked about the details of each food/beverage item, such as the serving size, container type (for beverages), amount of serving consumed, and any condiments or toppings. Pictures are used to help participants select the appropriate responses. The program then asks the participant to review a list of foods and beverages that are commonly forgotten by respondents, such as water or candy. After a final review, participants are offered one additional opportunity to report items. The last item asks the participant whether the reported consumption is representative of his or her usual daily intake. Supplement information may also be collected if specified by the investigator. The ASA24 instrument is offered in both English and Spanish. The most recent versions of the ASA24 system have been adapted for use with adults living in Canada (ASA24-Canada), with school-aged children (ASA24-Kids), and can also be used as a food record in order to allow for real-time data collection and for promoting dietary change. Efforts are underway to adapt the ASA24 for use with adults living in Australia [46].

In theory, the ASA24 offers the benefits of traditional telephone-based or in-person recalls while removing much of the expense. The ASA24 is available at no cost to researchers and is intended for self-administration by participants, eliminating the need for data entry or computation by trained dietitians. Some participant populations, however, may find it difficult to report their diet accurately using the system. This is particularly likely for individuals who are not accustomed to using a computer, are low in literacy, or who eat a lot of home-prepared dishes with many ingredients. The lack of a human interviewer to answer questions and probe for clarification may exacerbate these issues. Finally, more research is needed to document its measurement characteristics. Other web-based recall approaches include the Oxford WebQ [47], DietDay [48], and the National Cancer Institute's targeted instruments (the Fruit and Vegetable Scan and Block Fat Screener) [49].

A major advantage of food records and recalls is that they include a time stamp for every eating occasion. Therefore, these data can be used to construct dietary behavior variables such as number of meals and snacks per day, breakfast skipping, and the nightly fasting interval. Research in rodents [50,51] and sparse human data suggest that meal timing may be an important factor in human health [27]. For example, a recent (2016) analysis of 2413 breast cancer survivors found that women who fasted <13 hours per night had a 36% increased risk of breast cancer recurrence compared to women who fasted ≥ 13 hours (hazard ratio: 1.36; 95% confidence interval, $1.05-1.76$) [52]. Women who had longer nightly fasting duration also reported more sleep and had lower concentrations of hemoglobin A1c. Large cohorts that only have FFQ dietary data are not able to investigate these types of hypotheses.

C Dietary Indices

In the last few decades, the use of indices to capture overall dietary patterns has gained popularity [13,53]. A dietary index is based on either current nutritional knowledge (i.e., dietary guidelines/recommendation) or existing dietary patterns with some cultural aspects (i.e., the Mediterranean dietary pattern) and can be used to describe adherence to a specific overall dietary pattern. Some commonly used dietary indices include the Healthy Eating Index [54,55], the Diet Quality Index (DQI) [56], or for instance, on Mediterranean dietary pattern, such as the MedDietScore [57]. Typically, the assessment tool is a score based on the degree of adherence to the dietary pattern. The score is calculated by assigning a value to variables representing low (or high) intake of specific food items associated with the dietary pattern of interest [13]. The score is then used to examine the relationship between

the risk of a cause-specific morbidity or mortality and adherence to the dietary pattern. There is documentation on the reproducibility and validity of dietary patterns [54,58].

The use of dietary indices reflects a movement toward testing dietary interventions that focus on eating patterns rather than a single nutrient or type of food. A good example is the PREDIMED study that examined the health impacts of the Mediterranean diet (MeDiet), defined as the traditional dietary pattern found in the early 1960s in Greece, Southern Italy, Spain, and other olive-growing countries of the Mediterranean basin. Specifically, this study randomized 7447 men and women at high CVD risk into three diets: MeDiet supplemented with extra-virgin olive oil, MeDiet supplemented with nuts, and control diet (advice on a low-fat diet). During a median time of 4.8 years, both MeDiet dietary patterns showed significant reductions in the CVD and incident diabetes in relation to the control group [59]. In summary, the utilization of dietary indices will complement analyses of nutrients or food, and maximize the value of dietary pattern assessment in nutritional epidemiology [13].

D Vitamin/Mineral Supplement Assessment

Historically, less attention has been paid to measuring vitamin/mineral supplement use compared to food intake. However, assessing vitamin/mineral supplement use is important because supplement use per se is an exposure of interest for the risk of several chronic diseases [60–64]. In addition, supplements are used by approximately half of all Americans, so they contribute a large proportion of total (diet plus supplement) micronutrient intake [29,65–67]. Epidemiologic studies typically use personal interviews or self-administered questionnaires to obtain information on three to five general classes of multiple vitamins (multivitamins with or without minerals, stress supplements, antioxidant mixtures, and other mixtures, including multivitamins with herbals), single supplements, the dose of single supplements, and sometimes frequency and/or duration of use [68].

In a validity study comparing a self-administered assessment method to label transcription among 104 supplement users, we found correlation coefficients ranging from 0.1 for iron to 0.8 for vitamin C [68]. The principal sources of error were investigator error in assigning the micronutrient composition of multiple vitamins and respondent confusion regarding the distinction between multiple vitamins and single supplements. These results suggest that commonly used epidemiologic methods of assessing supplement use may incorporate significant amounts of error in estimates of some nutrients. In a subsequent study, we found that a similar inventory reporting system captured supplement use when compared to blood, toenail, and urine biomarkers [66,69]. In a marketplace that is becoming rapidly more complex, with vitamins, minerals, and botanical compounds combined in unusual mixtures at highly variable doses, the association of dietary supplements with disease risk is becoming increasingly difficult, but important, to assess [70].

E Use of Biomarkers in Nutritional Epidemiology

Dietary biomarkers have been critical to the advancement of nutritional epidemiology and have led to huge advances in our understanding of errors in self-reported dietary intake [32,71]. Biomarkers from blood, urine, stool specimens, or toenails can provide objective estimates of dietary intake and therefore circumvent problems associated with self-reported diet such as underreporting. Two major drawbacks of biomarkers are that (1) biological specimens are expensive to collect, store, and analyze in large studies and (2) suitable biomarkers have not been identified for all foods or nutrients. For example, there is no established biomarker for total fat or carbohydrate intake.

Dietary biomarkers fall into one of two general categories: recovery biomarkers and concentration biomarkers. Recovery biomarkers are those that have a known quantitative time-associated relationship between dietary intake and excretion or recovery in human waste (e.g., urine or feces) [72,73]. Concentration biomarkers (e.g., serum β-carotene for total fruits and vegetables) are responsive to diet and generally have a linear association with dietary intake [74,75]. However, these qualitative biomarkers are of limited usefulness in assessing overall or person-specific biases in self-report and cannot be used to estimate absolute intake in the same manner as recovery biomarkers [72,73].

Urinary nitrogen is an example of a recovery biomarker and is used to estimate protein intake. The measure is based on the following equation [76]:

$$\text{Reported protein intake (g)} = (\text{24-hour urinary nitrogen} + 2) \times 6.25\text{(g)}$$

Similar to a single 24-hour dietary recall, a single 24-hour urine collection does not reflect usual intake, but nitrogen intake has been shown to be less variable than protein intake such that only 8 days of collection are required compared to 16 days of dietary intake data to assess habitual protein intake. Although the collection of 24-hour urine is a tedious procedure, the method is readily accessible and comparatively inexpensive. In addition, other markers of dietary intake and intermediate risk markers may also be measured in the 24-hour urine that is obtained.

The "gold standard" for assessing energy intake is the use of doubly labeled water. Although this method actually measures energy expenditure (carbon dioxide output), it can be used as a measure of energy intake because energy intake and expenditure are approximately equivalent in weight-stable individuals. Doubly labeled water is water that has been "tagged" by replacing some of the hydrogen and oxygen with heavy isotopes (deuterium and oxygen-18). The participant ingests a dose of the doubly labeled water, and after a delay of a few hours, urine samples are collected to obtain a measure of the markers once they have reached equilibration with water in the body. As time continues, the deuterium will be eliminated as water and the oxygen-18 will be eliminated as water plus carbon dioxide. Thus, the rate of carbon dioxide production can be deduced by comparing the elimination of the two markers. Only one follow-up urine sample is required, and it can be collected up to a few weeks after baseline. Although it is expensive, this method has a relatively low participant burden and can accurately estimate energy intake and expenditure.

Energy expenditure can also be estimated using other methods, such as indirect calorimetry or accelerometers. In indirect calorimetry, a metabolic chart is used to measure the participant's oxygen intake and carbon dioxide output, which are then used to calculate the resting metabolic rate. A major disadvantage of this method is that it does not capture energy expended by movement or physical activity and is therefore inappropriate if free-living energy expenditure is of interest.

Accelerometers (e.g., the ActiGraph) are small electronic meters typically used to provide an objective measurement of physical activity. Worn on the hip during all waking hours over several days, these devices record movements during daily activity. Accelerometers can also be worn on the wrist, which can increase compliance, allows for 24-hour wear, and provide an objective assessment of sleep duration and quality [77]. By entering data for sex, age, and weight, investigators can apply algorithms to estimate the energy expended during each day or for the entire wear period [78].

The primary advantage of accelerometry is the objectivity of the data. Limitations to this approach include the pragmatic considerations involved in distributing and retrieving the monitors, the short assessment period (typically 1 week), the inability to discern type of activity being performed, and, to some extent, the cost of the devices. Recently, consumer-based trackers such as the Fitbit, Jawbone Up, and others have also been used by researchers to assess physical activity and energy expenditure. Validation studies indicate that fitness trackers can accurately measure steps but may be only moderately accurate for estimation of total daily energy expenditure [79–81]. Even so, the lower cost of these trackers, combined with their ability to provide continuous monitoring over weeks or months, supports their use in certain research contexts.

III STUDY DESIGNS USED IN NUTRITIONAL EPIDEMIOLOGY

Epidemiologic studies can be divided into two general types: observational and experimental. The three primary observational study designs are ecologic, case–control, and cohort studies. In human studies, the main experimental study designs are intervention trials or randomized controlled trials. An overview of these study designs in relation to nutritional epidemiology is given here.

A Observational Studies

1 Ecologic and Migrant Studies

Important hypothesis-generating studies have examined the relationship of national estimates of per capita supply of foods (e.g., dietary fat) with time-lagged rates of cancer or heart disease incidence or mortality [82–85]. These analyses strongly suggest that dietary fat intake increases risk while plant foods decrease risk of these major diseases. However, there are numerous problems with country-specific measures of dietary intake. First, estimates of per capita intake from food disappearance data are extremely imprecise and include nonhuman consumption uses such as livestock feed and manufacturing use of food or food end products (e.g., corn biofuel and soybean-based inks used in newsprint). Second, it is generally not feasible to control for other differences between countries (e.g., differences in physical activity levels or smoking prevalence). Finally, it is unknown whether the individuals within the countries that are exposed to specific dietary factors are the same individuals experiencing the disease.

Migrant studies have often shown that with a single move from less developed to Westernized countries, large and significant increases occur in risk of several chronic diseases such as breast cancer [86,87]. These changes occur rapidly, often after just one generation, as immigrants become acculturated to the diet and other habits of their new country [88]. Migrant studies offer strong evidence to support a major role for lifestyle and environment exposures as disease risk factors; however, few such studies have included pertinent dietary assessment to be able to properly address these questions [88].

2 Case–Control Studies

In a case–control study, individuals are identified and studied according to a single disease outcome. Specifically, individuals who have recently been diagnosed with a disease (e.g., colon cancer) are asked about their past

exposure to diet and other risk factors and often provide a blood sample. A comparable set of control individuals, usually drawn from the same population, are also enrolled in the study and are asked about their past exposures. The two groups (those with and those without the disease) are then compared for differences in dietary intake and other exposures. The major advantage of this design is that an entire study can be completed in just a few years with a smaller sample size than is needed for other study designs (it could be as small as 200 cases and controls). However, this study design can only answer questions about a single disease outcome.

In addition, these studies can introduce potentially serious biases. Two major concerns with case–control studies are recall bias and selection bias. In studies of chronic disease, investigators typically ask participants to recall behavior and other exposures (e.g., dietary intake) from the past 5–10 years, or even earlier. Bias can occur when cases recall exposure to potential risk factors differently than do controls. For example, past consumption of fatty foods might be more salient and easier to recall for individuals diagnosed with CVD than for healthy individuals. Selection bias occurs when controls agree to join the study because of an interest in health and are therefore more likely to exhibit healthy behavior (e.g., eat healthful diets and be physically active). The higher prevalence of healthy behavior in the controls appears to be associated with reduced risk of disease when actually it is associated with willingness to participate in a research study on health. Thus, control selection is an extremely important part of study design [1,89]. Furthermore, because cases are usually recruited relatively soon after diagnosis, unless remote diet is assessed, the dietary habits reported during the previous year (or a more recent time frame) represent dietary intake in the preclinical phase of the disease. Inferences from such data are not clear with respect to understanding diet–disease relationships.

Another problem with case–control studies is that biomarkers of diet (e.g., serum micronutrient concentrations) are potentially affected by the disease process and therefore may not be reliable indicators of long-term status (e.g., risk) in cases. As described in the following section, this problem is partially overcome in nested case–control studies.

3 Cohort Studies

The cohort study typically enrolls people who are free of disease, assesses baseline risk factors, and then follows the participants over time to monitor disease occurrence. The major advantage of cohort studies is that exposure to potential risk factors is assessed before the development of disease [1,89]. Therefore, exposures such as self-reported dietary intake or nutritional biomarkers from blood samples cannot be influenced by the disease process. In addition, cohort studies can examine many different exposures in relation to several different disease outcomes. A cohort study is generally a large enterprise because most diseases affect only a small proportion of a population, even if the population is followed for many years. These studies typically have sample sizes exceeding 50,000, can have a total cost in excess of $100 million, and require that the cohort be followed for 10 or more years [1,89]. Despite the cost and logistics, cohort studies have been useful in identifying diet–disease risk factors with ensuing recommendations for public health [90–95].

Because of the large size of these studies, the analysis of biologic markers (e.g., serum micronutrient concentrations) for all participants is prohibitively expensive. Therefore, cohort studies often archive (e.g., store) serum or plasma, white blood cells, toenails, DNA, or other biologic specimens for the purpose of conducting nested case–control studies in the future [96–98]. In a nested case–control study, a sample of cohort participants who developed a disease such as breast cancer (e.g., cases) is matched with other individuals in the cohort who did not develop the disease (e.g., controls). Cases and controls may be matched with respect to age, gender, and/or other important characteristics. This ensures that the sample of cases is comparable to the sample of controls with respect to potential confounding factors. Once cases and controls have been selected, biologic samples from these individuals are retrieved and analyzed to determine whether there are differences in prevalence of exposures (e.g., diet) between the two groups [99,100]. This can be an efficient and powerful study design that avoids many of the pitfalls of the classic case–control studies.

B Intervention Trials

Intervention trials prospectively examine the effect of an exposure randomly assigned by the investigators, such as a low-fat diet or a dietary supplement, on an outcome such as disease occurrence, risk factor for a disease, or a biomarker. An important consideration when designing these studies is the degree of dietary control needed. For example, depending on the hypothesis, the dietary intervention could be a controlled diet provided by the investigators, a vitamin supplement, or dietary counseling. The stringency of dietary control is determined partially by the expected size of the response (e.g., change in disease risk) and the length of the treatment period required. If the required dietary treatment period exceeds several months, a controlled feeding study is usually not logistically or financially viable.

It is also important to note that with the exception of dietary supplement intervention trials, most dietary

interventions are not double-blinded. If the study compares a low-fat eating pattern to usual diet, for example, participants will know to which arm they have been randomized because they are being asked to make specific dietary changes. Furthermore, as with any intervention trial, one must account for "drop-in" and "dropout" rates. Some study participants may find the required intervention activities too burdensome, so they may drop out or be less than 100% adherent to study activities [101,102]. Control participants, on the other hand, even if they are asked not to change their diet or take any new dietary supplements, may begin new dietary patterns that could be similar to the intervention. Both drop-in and dropout phenomena can diminish the amount of contrast between the intervention and comparison groups, thereby attenuating any effects of the intervention.

In an intervention trial, the random assignment of participants to the control versus the intervention group means that participants with predisposing conditions or unmeasured factors that might influence the outcome are equally likely to be randomized into the intervention or the control group. Therefore, there is little or no confounding in randomized intervention studies [89]. In addition, random allocation of the exposure eliminates the potential for selection bias and recall bias. However, such trials are generally expensive and labor intensive. Therefore, randomized trials are only conducted for important public health questions where the observational data are suggestive but a controlled experiment is needed prior to issuing public health recommendations. Randomized trials are the only epidemiologic study design in which cause and effect may be concluded [1,89].

IV INTERPRETATION OF CAUSE AND EFFECT IN NUTRITIONAL EPIDEMIOLOGY

Given that nutritional epidemiology is the study of dietary intake and its association with disease risk, we must use careful scientific judgment in determining when the strength of the evidence supports a causal link between the exposure and the outcome. When assessing causality, important considerations include: (1) the main measure of association used in epidemiologic studies; (2) the major alternative explanation for an observed association in observational studies, which is confounding; and (3) methods for assessing causality in studies of associations. Other important considerations include: (1) biological plausibility, (2) temporal association, (3) the strength of the association, (4) dose−response relationship, and (5) consistency with other studies [1,89].

A Measures of Association

The most commonly used measure of association between dietary intake and disease risk is relative risk (RR). The RR estimates the magnitude of an association between the dietary exposure and disease and indicates the probability of developing the disease in the exposed group relative to those who are not exposed [1,89]. For example, an RR of 1.0 indicates that the incidence of disease in both exposed and unexposed groups is the same. An RR greater than 1.0 indicates a positive association. For example, an RR of 2.0 between dietary fat and colon cancer indicates that individuals eating a high-fat diet are twice as likely to develop colon cancer as those eating a low-fat diet. RRs less than 1.0 are typically considered protective. An RR of 0.5 for the association of vegetable intake with colon cancer risk indicates that among individuals with diets high in vegetables, the risk of colon cancer is approximately half compared to those with diets low in vegetables. Often, RRs are given for the highest category of intake (e.g., highest quartile of fat or vegetable intake) in comparison to the lowest category of intake.

Given the degree of measurement error in dietary intake estimates, RRs in nutritional epidemiology rarely exceed 3.0. RRs are typically presented with their associated CI (e.g., RR 2.0, 95% CI of 1.3−2.9), which provides information on the precision of the point estimate (e.g., the RR). Specifically, it is the range within which the true point estimates lie with a certain degree of assurance. Typically, 95% CIs are given, which correspond to the traditional test of statistical significance, $p < 0.05$, meaning that there is less than a 5% probability that the findings occurred by chance. A 95% CI that does not include the null value (1.0) is, by definition, statistically significant at the $p = 0.05$ level. The width of the CI also provides information about the variability in the point estimate, which is primarily a function of sample size. Therefore, the wider the CI, the more variability in the measure, the smaller the sample size, and the less confidence we can have that the observed point estimate is the true point estimate.

It is important to separate the strength of an RR from its public health relevance. For example, a large RR (e.g., RR = 5.0) might be observed between a specific food and a risk of disease. However, if consumption of that food is rare, then its overall influence on the population's total morbidity or mortality will be minimal. Conversely, an RR of 1.5 might be very important from a public health perspective if the dietary exposure is common. Once RR estimates are used to determine the strength of association, then projections of the consequence of an exposure on public health (termed population attributable risk) become important in the development of policy and allocation of resources. For example, the consistent observations from observational studies that trans-fats were associated with unfavorable serum lipid profiles and CVD led to new food labeling laws requiring that trans-fats be listed among the "Nutrition Facts" [8].

B Confounding

Confounding occurs when an observed association between dietary intake and disease is actually due to another factor (e.g., physical activity) that is highly correlated with dietary intake and disease risk [1,89]. Confounding is a critical concept in nutritional epidemiology because it is plausible that people who have healthful diets are likely to differ from those who did not choose healthful diets with regard to other exposures (e.g., physical activity) [13].

For example, a population-based study among 1449 adults observed that those who used vitamin/mineral supplements were more likely to be female, older, better educated, nonsmokers, regular exercisers, and to consume diets higher in fruits and vegetables and lower in fat [14]. We also found previously unreported associations of supplement use with cancer screening, use of other chemopreventive agents (e.g., aspirin), and a psychosocial factor (belief in a diet−cancer connection). These relationships could confound studies of supplement use and cancer risk in complex ways. For example, male supplement users were more likely to have had a prostate-specific antigen test, which is associated with increased diagnosis of prostate cancer [103]. Therefore, supplement use could spuriously appear to be associated with increased incidence of prostate cancer.

The observed relationship between supplement use and belief in a connection between diet and cancer is especially interesting. Health beliefs influence cancer risk through behavior such as diet and exercise. For example, in a previous prospective study, we found that belief in a connection between diet and cancer was a statistically significant predictor of changes to more healthful diets over time [104]. In cohort studies, the increasing healthfulness of supplement users' diets and other health practices over time could result in a spurious positive association between supplement use and chronic disease risk.

It is important to note that in studies in which nutrient intake is summed from foods and supplements, the intake of micronutrients in the highest exposure category is often too high to be obtained from food and reflects supplement use. Therefore, studies of nutrient intake may also be confounded by the relationship between supplement use and healthful lifestyle. In these studies, consistency of findings for the nutrient from foods and vitamin supplements separately would increase confidence that an observed association was not confounded by supplement users' healthful lifestyles.

In theory, statistical adjustment in analyses for participant characteristics and major health-related behavior controls for the effects of confounding factors are important. However, the absence of residual confounding cannot be ensured, especially if other important confounding factors are unknown, not assessed, assessed with error, or not included in the analyses.

C Evidence of Causality

Epidemiology is the study of associations, and statistical methods provide the means to conduct hypothesis testing to quantify the association. However, it is important to note that the existence of a statistically significant association does not indicate that the observed relationship is one of cause and effect. For any observed association, the following questions should be considered:

- How likely is it that the observed association is due to chance?
- Could this association be the result of poor study design, poor implementation, or inappropriate analysis?
- How well do these results meet other criteria of causality, as given in Table 7.2 [1]? Specifically, is the association weak or strong? Is there a plausible biologic mechanism? Did the exposure precede the outcome? Is there a dose−response relationship?
- How well do these results fit in the context of all available evidence on this association? Causality is supported when a number of studies, conducted at different times, using different methods, among different populations, show similar results. Note that true causality can only be inferred within the context of an experimental study, a randomized controlled trial.

In a field characterized by as much uncertainty as nutritional epidemiology, it is rare for a cause-and-effect relationship to be considered unequivocal. However, lack of complete certainty does not mean that we should ignore the information that we have or postpone the action that appears needed at a given time [1,105,106]. It merely means that we exercise prudence and thoughtful consideration before acting on epidemiologic evidence.

V OBSTACLES TO FINDING ASSOCIATIONS OF DIETARY INTAKE AND DISEASE RISK

Here, we review the major obstacles to epidemiologic research, including error in exposure assessment and limitations of study designs.

A Assessing the Reliability and Validity of Measures of Dietary Intake

Reliability is generally used to refer to reproducibility, or whether an instrument will measure an exposure (e.g., nutrient intake) in the same way twice on the same respondents.

TABLE 7.2 Criteria for Judging Whether Observed Associations Between Diet and Disease Risk are Causal

Criteria	
Strength of the association	The stronger the association, the less likely that it is due to the effect of an unsuspected or uncontrolled confounding variable.
Biological credibility	A known or postulated biologic mechanism supports causality. However, an association that does not appear biologically credible at one time may eventually prove to be so. Implausible associations may be the beginning of the advancement of knowledge regarding mechanisms.
Consistency	This criterion requires that the association be observed in several types of studies—e.g., in more than one population and using different study methods.
Specificity	This is the degree to which one factor predicts the frequency or magnitude of a single disease.
Time sequence	The exposure of interest must precede the disease outcome by a time span consistent with known biologic mechanisms.
Dose–response	Evidence for a dose–response relationship (i.e., increased risk associated with increased exposure) is considered supportive of causality.

Source: Adapted from C.H. Hennekens, J.E. Buring, Epidemiology in Medicine, Little Brown, Boston, MA, 1987 and M.L. Neuhouser, B. Thompson, C.C. Solomon, Higher fat intake and lower fruit and vegetables intakes are associated with greater acculturation among Mexicans living in Washington state. J. Am. Diet. Assoc. 104 (2004) 51–57.

Validity, which refers to the accuracy of an instrument, is a considerably higher standard. Generally, a validity study compares a practical, low-cost measurement method (e.g., an FFQ) with a more accurate but more burdensome or expensive method (e.g., food records). Reliability and validity are typically investigated by means of statistical measures of bias and precision [12].

In a reliability study, reproducibility is assessed by comparing mean intake estimates from two administrations of the instrument in the same group of respondents. If an instrument is reliable, the mean intake estimates should not vary substantially between the two administrations. In a validity study, bias is generally assessed by comparing the mean estimates from the instrument of interest to those from a criterion measure (e.g., a "gold standard" instrument) in the same respondents. This comparison allows us to determine whether nutrient intake estimates from the first instrument appear to be generally under- or overreported in comparison to the criterion measure [12,13]. Bias is particularly important when the objective is to measure absolute intakes for comparison to dietary recommendations or some other objective criteria. For example, bias is critical when estimating how close Americans are to meeting the dietary recommendation to eat five servings of fruits and vegetables per day.

Precision is concerned with whether an instrument accurately ranks individuals from low to high nutrient intakes, which is typically the analytic approach used to assess associations of dietary intake with risk of disease [13]. In this situation, bias in the estimate of absolute intake is not important as long as precision is good. In a validity study, precision is the correlation coefficient between nutrient intake estimates from the instrument of interest in comparison to a criterion measure. Often, dietary assessment studies also assess validity by ranking nutrient intake estimates, dividing them into categories (e.g., quartiles), and comparing these to similar categories calculated from another instrument. However, classifying a continuous exposure into a small number of categories does not reduce the effects of measurement error [12] and, therefore, this analysis does not yield additional information beyond that provided by correlation coefficients.

It is important to know that an instrument can be reliable without being accurate. That is, it can yield the same nutrient estimates two times and be wrong (e.g., biased upward or downward) both times. Alternatively, an instrument can be very reliable and can consistently yield an accurate group mean (e.g., unbiased) but have poor precision such that it does not accurately rank individuals in the group from low to high in nutrient intake. Reliability is easy to measure, and nutrient correlation coefficients between two administrations of the same FFQ are generally in the range of 0.5–0.8 [13]. Estimates of reliability give an upper boundary to the accuracy of an instrument. Although a high reliability coefficient does not imply a high validity coefficient, a low reliability coefficient clearly means poor validity.

1 Sources of Error Associated With Self-Reported Dietary Intake

Regardless of the approach used, self-reported dietary intake is inherently subject to several sources of error (Table 7.3). Sources of random error that are common across dietary assessment methodologies include error in estimation of portion sizes, forgetting to report foods or beverages, and mistakes in reporting. Bias can occur when under- or overreporting of intake is related to some characteristic of the participants. For example, obese

TABLE 7.3 Sources of Error and/or Bias in Dietary Intake Estimates

Source of Error	Type of Error	Reason for Error	FFQ	Food Record	Food Recall
Participant	Memory	Unable to recall food consumption. This error increases with interval of memory required.	X		X
	Frequency judgments	Respondent has cognitive difficulty accurately providing this information. May be a particular problem in low-literacy respondents.	X		
	Question comprehension	Respondent may not understand what foods are being asked about, understand the frequency categories, or be able to estimate portion sizes.	X		
	Response errors	Respondent mistakenly codes incorrect frequency or skips questions.	X		
	Portion size errors	Respondent cannot conceptualize reference portion size or his or her own portion sizes.	X	X	X
	Social desirability bias	Respondent unintentionally (or intentionally) misrepresents dietary intake in order to please investigators. For example, obese participants may underestimate intake.	X	X	X
	Fatigue/burden	Respondent alters normal food choices or omits some items to simplify record keeping.		X	
Investigator/ tool	Food list	Food list is too short or not appropriate for population being studied and therefore dietary intake data are incomplete.	X		
	Food groups	Food groups may not appropriately group foods by nutrient composition.	X		
	Portion sizes	The reference portion size may be too large or too small for a population such that it consistently over- or underestimates amounts of food consumed.	X		
	Categorization of frequencies	Loss of information by using close-ended categories (e.g., 2−4 times/week) instead of using an open-ended format.	X		
	Poor form design	Font is too small, skip patterns are not clear, or instructions are unclear.	X		
	Data collection errors	Scanning errors may occur. Data from incomplete FFQs are used in analysis.	X		
	Database	Database may have incorrect nutrient values, incomplete nutrient values, or be missing important exposures altogether (e.g., isoflavones).	X	X	X
	Programming errors	Nutrient analysis program may contain errors.	X	X	X
	Estimation	Investigator relies on estimates to quantify nutritional intake. With food records, there is no opportunity to probe participant for additional information.		X	X
Other	Seasonal variation	It may not be possible to adequately report average intake of foods where intake varies markedly over seasons.	X		
	Unusual dietary patterns	Respondents with unusual eating patterns (e.g., liquid diets) may not be able to accurately report dietary patterns.	X		
	Short time period	Short duration of reference period results in a "snapshot" that may not be representative of respondent's usual intake.		X	X
	Intervention-associated bias	Respondents in an intervention are more likely to report socially desirable responses.	X	X	X

individuals tend to underreport their energy intake to a greater degree than normal-weight individuals [107]. Another concern is social desirability bias, which is the tendency to respond in a pleasing way. For example, participants enrolled in a program to increase fruit and vegetable intake may consciously or subconsciously overreport their intake of these foods. Susceptibility to social desirability bias varies among individuals and has been associated with differential error in the reporting of energy intake [108]. Furthermore, research indicates that dietary interventions introduce reporting bias toward the more desirable responses [109]. Finally, food records, food recalls, and FFQs are all subject to limitations in the food database and errors in the programming used to quantify nutrient intake.

2 Measurement Error Specific to FFQs

The FFQ form is a major source of error due in part to the limitations inherent in the following commonly used components: closed-ended scannable response options, limited food list (generally approximately 100 items), inadequate food composition information, and instructions for respondents to report their average intake over long periods of time, such as one year.

Studies comparing FFQs with records or recalls are often called validation studies. The theory behind this type of study is that the major sources of error associated with FFQs are independent of those associated with short-term dietary recall and recording methods, which avoids spuriously high estimates of validity resulting from correlated errors. As summarized by Willett [13], errors unique to the FFQs include the restrictions imposed by a fixed list of foods, perception of portion sizes, and the cognitive challenge of assessing frequency of food consumption over a broad time frame. However, it is clear that many sources of error (e.g., underestimating portion sizes) are common to all self-report assessment instruments.

3 Measurement Error Specific to Food Records/Recalls

Unlike FFQs, food records and recalls are open-ended, do not depend on long-term memory, and allow for measurement of portion sizes. In addition to the sources of error that are common across self-reported dietary assessment methods, food records are also prone to bias that results when participants change their eating habits during the assessment period. This may occur as the result of social desirability bias (e.g., respondent decreases intake of unhealthy foods to avoid having to report these items) or due to burden and fatigue (e.g., respondent begins eating fewer foods or more simply prepared dishes to make it easier to record intake). Like food records, food recalls are typically open-ended. However, recalls are usually collected without advance notification. Therefore, participants cannot change what they eat retroactively and the instrument itself should not affect food intake, although misreporting due to social desirability bias is still possible. Both recalls and records are subject to coding errors because scannable forms are not typically used.

4 Comparison of Self-Reported Diet to Objective Biomarkers

Validity studies that compare self-report dietary instruments against one another are subject to certain limitations. They rely on the assumption that there will be little overlap between the sources of error and bias on the two instruments. This is unlikely to be fully true because some sources of error are common to all self-report assessments. For this reason, recent validity studies have focused on comparing self-reported diet to objective biomarkers, such as doubly labeled water. As described previously, the doubly labeled water method is a gold standard method that provides an accurate assessment of total energy expenditure (essentially equivalent to energy intake). A 2014 paper reported on pooled data from five large validation studies of dietary self-report instruments that used recovery biomarkers as references to clarify the measurement properties of FFQs and 24-hour recalls. Using an FFQ, the average correlation coefficients for reported versus true intakes for energy, protein, and protein density were 0.21, 0.29, and 0.41, respectively. Using a single 24-hour recall, the coefficients were 0.26, 0.40, and 0.36, respectively, for the same nutrients and rose to 0.31, 0.49, and 0.46 when three 24-hour recalls were averaged. The average rate of underreporting of energy intake was 28% with an FFQ and 15% with a single 24-hour recall, but the percentages were lower for protein. Personal characteristics related to underreporting were body mass index (BMI), educational level, and age. This project established that their self-reported energy and protein intake are poorly correlated with truth, the use of multiple 24-hour recalls substantially increases the correlations when compared with a single 24-hour recall, and BMI strongly predicts underreporting of energy and protein intakes [110].

5 Effects of Error in Dietary Intake Estimation and Measures of Disease Association

Error in dietary assessment can be of two types, with markedly different consequences. Random error refers to mistakes such as inadvertently marking the wrong frequency column, skipping questions, and lapses in judgment. These errors introduce noise into nutrient estimates such that our ability to find the "signal" (e.g., an association of dietary fat and breast cancer) is masked or attenuated (biased toward no association).

Systematic error refers to under- or overreporting of intake across the population (e.g., bias) as well as to person-specific sources of bias. For example, studies indicate that obese women are more likely to underestimate dietary intake than are normal-weight women [108,111,112]. Systematic error may result in either null associations or spurious associations. In one report, Prentice used data from FFQs collected in a low-fat dietary intervention trial to simulate the effects of random and systematic errors on an association of dietary fat and breast cancer, where the true RR was assumed to be 4.0 [113]. Assuming only random error exists in the estimate of fat intake, the projected (i.e., observed) RR for fat and breast cancer would be 1.4. Assuming both random and systematic errors exist, the projected RR would be 1.1, similar to that reported in a pooled analysis of cohort studies [114]. These results indicate that FFQs may not be adequate to detect many associations of diet with disease, even if a strong relationship exists [115,116]. In view of the error in dietary self-report, it is not surprising that results from diet–disease studies are often null or conflicting [113,117–120]. Because of cost or practical considerations, the use of objective biomarkers is not feasible in epidemiologic studies. One approach for large studies using self-reported diet assessment is to collect biomarker data on a subset of participants and use that data to develop calibration equations that reduce the "noise" component of dietary measurement error [117,119] and correct for subject-specific error in reporting that can reduce bias in diet–disease association studies [121].

B Limitations in Research Designs

1 Observational Studies

In studies of nutritional epidemiology, unique obstacles exist in finding clear and interpretable relationships between dietary intake and disease risk [106,118]. In roughly increasing order of importance, these obstacles include the following:

- Current or recent dietary intake may differ from intake over the time frame relevant to the development of disease, which will reduce our ability to find associations between diet and disease.
- Certain nutrient intakes within a population may not be highly variable. For example, energy from dietary fat in a population of postmenopausal women may only vary from 25% to 40%, resulting in inadequate range of disease risk to find an association with breast cancer. This situation is akin to assessing whether smoking causes cancer by studying men who smoke one pack per day in comparison to men who smoke one and a half packs per day. Minimal heterogeneity in exposures provides insufficient contrasts.
- Diet is a complex mixture of foods and nutrients, including many highly correlated compounds, making it difficult to separate the effects of any one compound from other dietary factors.
- Dietary intake may relate in a complicated manner to other risk factors such as hormonal status, obesity, or hypertension. These relationships (some of which may be in the causal pathway) make it difficult to appropriately control for confounding factors.
- Existing dietary self-report instruments include many sources of random and systematic error, both of which obscure our ability to find associations between dietary intake and disease risk.

An important point to consider is that most of the obstacles listed previously will limit or attenuate our ability to find associations between dietary intake and disease. For example, as shown in Table 7.4, an observed association of dietary fat intake with BMI might appear too small to be clinically important. However, if we assume that significant measurement error exists in our estimate of fat intake (e.g., a correlation of 0.30 between our measure and "true" intake), then the real association would be 4.0 BMI points per 10 g of fat intake, which is considerably more important. Therefore, studies showing weak or no associations between dietary intake and disease (e.g., null results) need to be interpreted cautiously.

Observational studies are frequently referred to as hypothesis-generating studies, which set the stage for testing in a large randomized trial with disease outcomes. However, many hypotheses that were well supported by

TABLE 7.4 Estimates of the Observed Association[a] Between Dietary Fat Intake (per 10 g of Fat) and BMI After Adjustment for Random Measurement Error in the Measure of Dietary Fat

Correlation Coefficient[b] Between the FFQ Estimate and "True" Fat Intake	Observed Increase in BMI for Every 10 g of Fat Consumed[c]
1.00 (FFQ is a perfect measure of fat intake)	4.0
0.70 (FFQ is a good measure of fat intake)	2.8
0.50 (FFQ is a weak measure of fat intake)	2.0
0.30 (FFQ is a poor measure of fat intake)	1.2

[a] $\beta_{observed} = \beta_{true} \times$ validity coefficient.
[b] Correlation coefficient from validity study comparing FFQ to multiple 24-hour recalls.
[c] Assume true regression coefficient from a multivariate model predicting BMI = 4.0.

laboratory and observational epidemiologic research have proven to be null when tested in a trial:

- Experimental and epidemiological data suggested that vitamin E supplementation prevents cancer and cardiovascular events. In a trial of 7030 patients with vascular disease or diabetes mellitus, long-term vitamin E supplementation did not prevent cancer or major cardiovascular events and resulted in some increase in the risk for heart failure [122].
- β-Carotene was hypothesized to reduce tumor incidence. However, two large trials to test the effect of β-carotene supplements on lung cancer incidence in smokers found that β-carotene supplementation increased the incidence of lung cancers as well as cardiovascular and all-cause mortality [123,124].
- B vitamins were believed to lower the risk of CVD. However, after 38 months of treatment and follow-up, supplementation with folic acid, B_{12}, and B_6 did not reduce total cardiovascular events among 3096 patients with coronary artery disease or aortic valve stenosis [125]. An analysis with long-term follow-up found that treatment with folic acid plus vitamin B_{12} was associated with increased cancer outcomes and all-cause mortality [126].
- A healthy diet, characterized to be low in fat and high in vegetables, fruits, and fiber, is hypothesized to reduce disease risk and disease progression in at-risk populations, such as postmenopausal women and breast cancer survivors. However, a trial of 48,835 postmenopausal women found that adoption of a diet high in vegetables, fruits, and whole grain and low in fat did not reduce the risk of CVD or risk of colorectal cancer over an 8-year follow-up period [127,128]. In a separate trial of 3088 breast cancer survivors, the adoption of a similar dietary pattern did not reduce additional breast cancer events or mortality during a 7.3-year follow-up period [129].
- Vitamin E and selenium were thought to reduce the risk of prostate cancer. Unexpectedly, a trial of 35,533 men found that dietary supplementation with vitamin E significantly increased the risk of prostate cancer among healthy men [130,131].

Although this list of null or negative trials is in no way comprehensive, it clearly illustrates that regardless of their size or duration, observational epidemiologic studies alone may not provide reliable information on the associations of dietary intake and disease.

2 Limitations of Clinical Trials of Dietary Intake and Disease Risk

Despite the many desirable features of dietary intervention trials, unique obstacles are present in these types of studies, as summarized here.

The costs of a long-term dietary intervention trial can be formidable. For example, the National Institutes of Health-sponsored Women's Health Initiative tested whether "low-fat eating pattern" would reduce the incidence of breast cancer, colorectal cancer, and coronary heart disease among 48,837 postmenopausal women in the United States [127,128,132]. The dietary intervention required participants to attend monthly sessions (run by specially trained nutritionists) for the first 18 months followed by quarterly classes for the remainder of the trial—approximately 8.5 years [133]. In addition, new intervention components were added to the trial to encourage adherence. The costs of implementing this type of intervention far exceed those required for comparatively simple pill−placebo trials or observational studies.

Maintenance of dietary adherence for a sufficient period of time to be able to ascertain clinical outcomes (e.g., disease risk) can be a formidable task. On the one hand, the greater the contrast in dietary intake between the intervention and control groups, the more likely the study will be able to detect an effect on the outcome. On the other hand, it is clearly more difficult to get participants to adhere to very strict or limited regimes, which can result in such poor adherence that the trial becomes futile [134]. Monitoring of dietary adherence typically requires use of self-reported dietary instruments, with their attendant weaknesses (discussed previously).

VI FUTURE RESEARCH DIRECTIONS

As is apparent from this overview of nutritional epidemiology, the major challenge is that of addressing random, systematic, and person-specific sources of error in dietary assessment. Only when well-designed validity studies clarify these sources of error we will be able to markedly improve our ability to draw valid inferences from epidemiologic studies of diet and disease.

A growing area of research concerns diet−gene and diet−environment interactions in the etiology and pathogenesis of chronic diseases. Despite the vigorous investigation of environmental causes of disease, it has long been recognized that not all persons exposed to the same risk factors will develop the associated disease [135,136]. For example, although it is well accepted that smoking will cause lung cancer, only 10−15% of smokers will be diagnosed with the disease in their lifetime. We are only beginning to understand the complexity of genetic susceptibility in the etiology and pathogenesis of common diseases such as coronary heart disease and cancer. If only a subgroup of individuals is sensitive to certain dietary exposures, the effect will be diluted and the association will be undetectable when the entire population is the focus of study. On January 20, 2015, President Obama announced the Precision Medicine Initiative (PMI) in his State of the Union address. The PMI Cohort Program

seeks to extend precision medicine to all diseases by building a national research cohort of one million or more US participants who contribute genetic, environmental, and lifestyle information over a long period of time [137]. This initiative has the potential to improve our understanding of individual susceptibilities and lifestyle factors. A second part of the PMI will advance the field of precision oncology by discovering unique therapies that treat an individual's cancer based on the specific abnormalities of their tumor [138]. Although focused on targeted therapies, this initiative offers considerable potential to expand this knowledge to nutrition. For example, a nested, case−control study of 265 postmenopausal breast cancer survivors found that among women whose tumor tissue was positive for insulin-like growth factor 1 (IGF1) receptor, stable or increased carbohydrate intake postdiagnosis was associated with a fivefold increased risk of recurrence [139]. This is the first study to suggest that it may be possible to personalize dietary recommendations for breast cancer survivors based on molecular characteristics of their primary tumor tissue.

Another exciting area is the use of new technologies to assess the influence of diet on the various "omics," such as proteomics and metabolomics [140−142]. These small molecules may prove to be more informative biomarkers of diet and diet−disease relationships than simple assessment of blood nutrients. For instance, recent evidence has implicated the gut microbiota, several distinct microbial ecosystems found within the gastrointestinal tracts of humans, to contribute in important ways to human health and disease. The "thrifty microbiome hypothesis" theorizes that the gut microbiota, specifically the relative proportions of two phyla (i.e., Bacteroidetes and Firmicutes), plays a key role in human energy homeostasis and impact the obesity epidemic [143−145]. This particular phenotype may introduce a survival advantage to its host in times of nutrient scarcity, promoting positive energy balance by improving metabolic efficiency and energy storage, and increasing efficiency of nutrient absorption [146]. However, in the presence of excess nutrients, obesity and obesity-related conditions may result [144,147]. Furthermore, potential factors that modify the relationship between the microbiota and risk of obesity are the host's external environment, geographic location, and behavioral factors (sedentary behavior and dietary patterns) [148].

In summary, despite the challenges in nutritional epidemiology and the measurement error issues that have impeded progress in the field, nutritional epidemiology studies have made important scientific contributions that have shaped public health policy and practice. To better understand the influence of diet on obesity-related health outcomes, efforts to reduce dietary measurement error through improved collection, evaluation, and analysis of consumption data are still urgently needed [11]. In addition, future research on diet and disease should focus on study designs that do not rely on self-reported diet as a measure of intervention adherence or an outcome. These research approaches include the use of randomized trial designs, the inclusion of dietary biomarkers, and the use of disease biomarkers such as insulin resistance. The challenge for the future is to decide which epidemiologic methods and study designs are most useful in studying chronic disease, determine which associations and the hypotheses derived from them are especially strong and worthy of pursuit, and finally to design randomized studies that are feasible, affordable, and likely to result in confirmation or refutation of these hypotheses [2].

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Chapter 8

Analysis, Presentation, and Interpretation of Dietary Data

Rachel K. Johnson[1], Deborah A. Kerr[2] and TusaRebecca E. Schap[3]

[1]*The University of Vermont, Burlington, VT, United States,* [2]*Curtin University, Perth, WA, Australia,* [3]*Alexandria, VA, United States*

I INTRODUCTION

Nutritional epidemiological studies play a critical role in relating dietary intake to risk of disease. These investigations often require gathering dietary intake data from large cohorts of people, which must then be translated into a usable format for analysis. This chapter focuses on research applications for the analysis, the examination of dietary data to determine the nutritional composition of participants' diets; presentation, the communication of the data and results in a logical format, such as comparing the results to a standard; and interpretation, the translation of the data and results—what do the data really tell us? The first step in planning a research study is to define the research question. The dietary assessment method and the type of analysis undertaken will be largely determined by the research question being addressed and the dietary assessment method that is best suited to the research question. How best to minimize measurement error through data collection and analysis should be one of a researcher's key considerations. In Chapter 1, Dietary Assessment Methodology, the advantages and disadvantages of dietary assessment instruments were outlined in detail. While ideally, the research question will drive the choice of method, but constraints of funding, research staff availability, and participant burden also influence choices in methods. With future advances in technology, it may be possible to overcome some of these limitations to dietary assessment so that more detailed methods can be undertaken without being limited by cost and participant burden [1].

II ANALYSIS OF DIETARY DATA

The methods most often used to obtain dietary intake information for research investigations include 24-hour dietary recalls, dietary records or diaries, and food frequency questionnaires (FFQs). The 24-hour dietary recalls and dietary records provide detailed descriptions of the types and amounts of foods and beverages consumed throughout a specified period of time, normally 1−7 days. The FFQ provides a less detailed list of selected foods and the frequency of their consumption in the past. See Chapter 1, Dietary Assessment Methodology, for further description of these methods. The data received must then be analyzed to determine the total intake of nutrients or food components consumed by each study participant.

A Preparation for Analysis

Prior to data analysis, the data need to be checked for missing data and data entry errors. Technology has helped a great deal with reducing data entry errors and ensuring there are no missing data (e.g., forcing a response on an electronic FFQ), but there are still areas where the researcher must make decisions on what to enter for analysis and ensure data cleaning has occurred before the analysis is undertaken.

The first step in this process is to check the participants' data for completeness. For interviewer-administered 24-hour dietary recalls, self-administered 24-hour recalls such as the ASA24, or computer-based FFQs, data may be coded as complete or incomplete. Thus making it simple for research staff to identify and exclude incomplete data. Food records, both digital and paper-based FFQs require thorough review to identify missing data.

Food records should be verified for missing data and research staff may contact participants to ask for details on food types and amounts in effort to improve the quality of the data [2]. Ideally, record checking should be done in-person with the participant so that portion size aids

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may be shown. Alternatively, contacting the person by telephone may also be done to verify items recorded. Image-based methods also require confirmation to ensure the participant has not missed taking images of any food or beverage during the recording period and to verify the content of the images. Research staff who are familiar with food composition, age-specific foods (e.g., foods commonly consumed by toddlers), ethnic or regionally specific foods, and the food database system being used for analysis, as well as the format in which foods need to be entered are critical for this review and verification step. This requires knowledge of the brand names along with the generic equivalent the food may be listed as in the food composition database [3]. In addition, with technology-based dietary assessment methods becoming more available, training in technology is becoming increasingly important. Ideally, the research staff should have qualifications in nutrition and dietetics and have undergone advanced training in dietary assessment. It is recognized, however, that not all graduates may have training in this important competency for dietetic professionals [4]. Although complete checking of records is ideal, there may be situations in which some details are missed or the participant is unable to provide the level of detail the researcher would like.

The task of undertaking 24-hour recalls or food records can become tedious for participants. When the researcher probes for more details, some participants may become concerned that they have not done a good job, whereas others may become bored and lose interest in the task [1]. The researcher must to be able to balance the need for detail with the burden placed on the participant, particularly when working with adolescents and young adults who may have a low tolerance to some methods [5,6]. In addition, with food records, there may be incomplete days of recording or participants may have been unwell and not eaten. For some participants, no amount of probing will result in a quality record due to a consistent lack of detail of the foods consumed. The researcher should make a decision if he or she considers the record suitable for analysis.

B Rules for Data Entry

When entering data, rules for data entry should be set up to ensure a consistent approach is applied to all records. This is particularly important when a team of researchers is entering the data. The researcher needs to be able to translate the food or drink reported to the best match in the food database. These decisions need to be recorded along with what to do about missing data so that a standardized approach is used across all data collected. In some situations, participants may not know the composition of the food or drink consumed. This commonly occurs with meals eaten outside the home or where they have not been involved in the food preparation. In this case, it is important to standardize the rules and decision making on what food is considered the best match for a food selected from the food database. For databases such as the Food and Nutrient Database for Dietary Studies used in the "What We Eat in America" (WWEIA) 24-hour recalls, for most foods there is an option to select a food code "not (further) specified" (NFS or NS) when the participant is unable to provide details of the food eaten. If the quantity of food eaten is not known, then "quantity not specified" may be chosen. These are known as defaults used for coding. Because these are based on usual consumption patterns from survey data, the use of default codes introduces more error; thus, their use should be minimized [7].

C Choosing a Nutrient Composition Database

A variety of computer-based food composition databases and nutrient computation systems are available in which the foods can be entered directly by name and computation of nutrient values is automated. The food composition databases are referred to as either the reference or user database. Selection of the correct food composition database is critical because the foods in the database must be appropriate to the geographical location and the ethnicity of the population being studied. Stumbo [3] and Buzzard and colleagues [8] summarize key points for selecting dietary assessment systems; e.g.: (1) Does the databases contain all the foods and nutrients of interest? (2) Is the database complete for nutrients of interest? (3) Do the food descriptions included in the database provide adequate specificity to accurately assess food components of interest? and (4) What quality control procedures are used to ensure the accuracy of the database? The accuracy of the data obtained from these systems will differ, depending on the following factors:

Updating of the database. New foods are constantly being introduced in the market, so the best databases are updated often to keep up with these changes. Virtually all databases use the US Department of Agriculture (USDA) Nutrient Database for Standard Reference (SR) as their primary source of nutrient data. The 2010 version, Release 28 (SR28), contains data on 8789 food items and up to 150 food components [9]. Nutrient profiles were added for new foods and existing nutrient profiles were updated in this release. A focus was to monitor those foods which are major contributors of sodium to the diet. Although the database may not include information on all food products currently in the marketplace, specific criteria

have been established for evaluating foods to ensure the data are as accurate as possible [10,11]. Many databases also add information from specific food manufacturers to provide information on name-brand foods not available in the SR.

The numbers and types of food items available. This is particularly important for recalls and dietary records or for FFQs containing write-in sections in which all foods must be assigned nutrient values. In regions with ethnocultural diversity, special care must be taken when selecting databases [12,13]. The databases that contain a variety of ethnic foods will provide greater accuracy and will require less manual entry of nutrient values for foods. The researcher must have knowledge of the naming convention and the search strategy required when using food composition databases [3]. Many food names can be ambiguous or spelled in a number of different ways—e.g., in the United States, ketchup versus catsup versus tomato sauce in England and Australia [3]. Pennington [14] summarized a number of useful examples of food items with more than one meaning and food synonyms.

The ability to add foods or nutrients. This is most important for those investigations in cohorts with multiethnicity [12,15,16] or when there is a high tendency for the participants to include restaurant foods that may not be included in the database. The trend is for greater consumption of food away from home [17], especially for teenagers and young adults [18]. This has implications for the accuracy of dietary assessment because when a restaurant-specific food is not available in a database, it may be difficult in some situations for participants to identify the contents and amount of food they have consumed. The ability to adapt or add recipe information should also be available. For example, if a participant reports homemade beef stew for lunch, the database should allow the coder to either add or delete ingredients from an existing recipe or add a new recipe to the file. The decision as to what foods to add to the database may depend on the research question and how important the composition of these foods may be to the study outcomes. Otherwise, choosing a similar food or an NFS item may be appropriate. There are increasing numbers of functional foods that may not appear in the database. Again, it depends on the study objectives as to how critical it is to add these foods or adjust the composition of existing foods.

The ease of data entry and analysis. Systems should be easy to use to avoid unnecessary coding errors. Entry of products by name, particularly brand names, should be available. Some databases, such as the Food Intake Analysis System [19], which is a nutrient analysis software program, offer default options. These choices provide average estimations of foods for which exact information is not known. For example, if a subject had chicken breast but was not sure of the cooking method or the serving size, the coder can choose the default option instead of making guesses. These options can help decrease differences in nutrient intake values caused by multiple coders or data entry technicians.

The nutrients available. Not every database contains all nutrients. A database may be limited to those nutrients available on the Nutrition Facts Panel or have as many as 65 nutrients/food components such as in the USDA Food and Nutrient Database for Dietary Studies. Some contain more accurate data for particular nutrients using analytically derived values while others may impute nutrient values. Systems should be evaluated for the accuracy of the nutrient values that are being studied.

The handling of missing nutrient values. Missing values exist if the food has not been analyzed for all food components [4]. If a specific nutrient value is unknown for a particular food, the way the database handles the missing information may affect the accuracy of the nutrient information. Some systems impute values, whereas others simply use a value of zero. An imputed value is almost always a better estimation [20]. However, imputing nutrient values is a labor-intensive task and requires nutritionists with knowledge of data evaluation and imputing procedures. Therefore, caution must be taken when using databases with imputed values.

The handling of dietary supplements. In studies where total nutrient intake is of interest, a database that includes dietary supplements will be needed. For example, in a study examining the relationship between calcium intake and bone health, total calcium intake from all sources including dietary supplements and calcium-fortified foods will be necessary. Merging data from a dietary supplement database with data from the food nutrient database may be necessary. The assessment of dietary supplement use is presented in detail in Chapter 2, Assessment of Dietary Supplement Use.

Standardizing dietary assessment. The emergence of large multicenter trials that may span across countries has presented challenges for nutrient analysis of dietary data. Because food composition may differ markedly, how best to pool nutrient databases must be addressed. The European Prospective Investigation into Cancer and Nutrition (EPIC) nutrient database project is an example of how researchers have attempted to standardize nutrient databases across 10 European countries [21]. A total of 26 priority nutrients were identified by the researchers along with procedures for food matching across counties.

D Statistical Approaches to Data Checking

Statistical packages such as IBM SPSS Statistics [22] and SAS [23] are useful for checking nutrient data for errors prior to undertaking analysis. Once data entry is completed, various tests should be undertaken to ensure data entry errors have not occurred. Some laboratories will perform "double entry" by two researchers of all records as a way to minimize data entry errors. Once this is complete, using the compare function in statistics packages, such as SAS, can be useful to identify where data entry errors have occurred. Additionally the explore function in SPSS or PROC UNIVARIATE in SAS checks for outliers or extreme values in the data. In general, results that fall well outside the mean should be further explored to determine if they are true outliers or a data entry error. It is recommended that all nutrients be examined for outliers because some data entry errors may be nutrient specific. For example, an outlier in β-carotene may have occurred if the serving size of carrots was entered incorrectly, but this may have little impact on energy (calorie) intake. Some researchers omit data from records with implausible energy intakes but this must be approached carefully as the application of arbitrary cutoffs may result in the loss of a substantial number of subjects [24]. Researchers may establish a priori high and low sex-specific cutoff points for energy intake and exclude data outside of the cutoff point. For example, in one study of pregnant women the researchers excluded reports with a calculated daily energy intake of <1000 and >5000 calories/day [25].

E Factors Affecting Food Composition Databases

When computing nutrient intake from food consumption data, it is assumed that the nutrient quality and content of certain foods are virtually constant and that what is consumed is available for use. However, we know that this assumption is not totally correct. There are various reasons why the actual value of a consumed nutrient may differ from the calculated value. The level of certain nutrients in foods may be affected by differences in growing and harvesting conditions (e.g., selenium [26,27]), storage, processing, and cooking (e.g., vitamin C [28]). Databases attempt to account for some of the differences by increasing the data banks to include preparation methods, cuts of meat, and specific manufacturers for processed food. For example, if chicken is entered into the database, the coder may have approximately 455 items to choose from. This large number includes name brand foods, particular pieces of chicken available (e.g., breast or thigh), and cooking methods (e.g., baked or fried, cooked with skin on or off, and skin eaten or not). Because so many choices are offered, recalls and records should be as detailed as possible to provide enough information to make an accurate selection.

The use of controlled feeding trials in a study, such as the Dietary Approaches to Stop Hypertension (DASH) trial [29], can help alleviate some of the differences between the calculated and the actual nutrient values of food. The DASH trial was a multicenter study designed to compare the effects of dietary patterns on blood pressure. The subjects were asked to consume only foods prepared by the research centers. Food procurement, production, and distribution guidelines were set and strictly adhered to at all sites to ensure that menus consistently met nutrient goals. For example, food items were given specific purchasing sizes, detailed descriptions, or defined brand names to ensure that all site recipes were of uniform composition [30]. Menu items were analyzed in a laboratory to obtain nutrient content values [31]. When possible, foods can be obtained from central suppliers to further eliminate any differences in nutritional content of foods due to regional variations in a study of this type.

The diet as a whole can also affect the availability of some nutrients. For example, phytic acid may decrease the availability of iron [32]. Computer-based analysis programs do not generally examine the overall diet and cannot determine how nutrient−nutrient interactions may affect availability. For example, iron is a mineral for which intake is not a good marker for availability. The absorption of iron is influenced by the following components: (1) the source of iron (more heme iron is absorbed than nonheme iron), (2) the iron status of the individual (decreased iron stores increase absorption), and (3) the overall composition of the meal. These components play a role in determining how much of the iron consumed is available to the body [33,34]. In turn, iron consumption can also affect the absorption of other nutrients, such as zinc. Nutrient−nutrient interactions can greatly determine how well a calculated nutrient value represents the actual available amount of a nutrient.

Other factors that should be taken into account are drug−nutrient interactions and those people who may be malnourished or suffer from malabsorption. For example, the elderly are more likely to have a decreased ability to absorb vitamin B_{12} than are younger adults. The elderly population is also at higher risk for drug−nutrient interactions because they are often prescribed many medications. Researchers must be aware of any illnesses or medications taken by subjects that could interfere with nutrient absorption.

Although food composition databases are increasingly becoming more accurate and may be closer to actual values of energy intake than laboratory analysis [31], they cannot provide exact measurements for all nutrient intakes. Furthermore, even if these values are determined to be

accurate, intake does not necessarily mean the nutrient is available for use. To obtain more accurate information on nutrient status, other methods, such as external reference biomarkers, should be employed. Also, familiarity with the participants' diets is essential for more accurate calculations. This includes, but is not limited to, factors such as dietary supplement use, medications used, the presence of diseases or illness, as well as special diets that participants may be following (e.g., vegetarian or weight loss). The decision to include or exclude individual dietary data will depend on how these factors may affect the study outcomes.

F Dietary Pattern Analysis

There is increasing interest by researchers in examining whole foods and dietary patterns rather than only single nutrients [35,36]. Many countries use a food-based approach to dietary guidelines, so examining how the diet compares to dietary guidelines may be of interest to researchers and nutrition policy makers. Diet quality indexes (DQIs) are also widely used and are able to quantify the risk of some nutrition-related health outcomes [37].

In reviewing the indexes of overall diet quality, Kant [38] found that there were three major approaches to the development of indexes: (1) derived from nutrients only, (2) based on foods or food groups, and (3) based on a combination of nutrients and foods. The definition of "diet quality" differs based on the attributes chosen by the investigators of each index [38], so the index chosen will depend on the needs of the study. The indexes based on nutrients only tend to consider consumption as a percentage of one of the nutrient-based reference values of the dietary reference intakes (DRIs), such as the recommended dietary allowance (RDA), as a marker for diet quality. Those based on food groups and dietary patterns examine the intake patterns of foods to identify patterns associated with adequacy and positive health outcomes [38].

Although numerous tools are available for examining overall diet quality and dietary patterns, those most commonly applied are based on the combination of nutrients and foods. These indexes, including the Healthy Eating Index−2010 (HEI-2010) [39], the DQI [40,41], the Diet Quality Index−International (DQI-I) [42], the alternate Mediterranean diet (aMED) score, and the DASH [43−46], use dietary guidelines [47] and food selection guides to score the overall diet. An update of the HEI to align it with the 2015−2020 Dietary Guidelines for Americans can be expected. Patterson et al. [41] were among the first to relate diet quality to the Dietary Guidelines for Americans. The DQI was based on dietary recommendations from the 1989 National Academy of Sciences publication *Diet and Health*, in which intakes were stratified into three levels for scoring. These points were summed across eight diet variables to score the index from zero (excellent diet) to 16 (poor diet). Haines et al. [40] revised the index in 1999, now called the Dietary Quality Index−Revised (DQI-R) [40], to reflect the updated guidelines.

The DQI-R incorporates both nutrients and food components to determine diet quality. It is based on 10 components, with a 100-point scale; each component is worth 10 points (Table 8.1). Components are based on total fat and saturated fat as a percentage of energy; milligrams of

TABLE 8.1 Diet Quality Index−Revised

Component	Maximum Score Criteria[a]	Minimum Score Criteria[a]
Total fat (% of energy intake)	≤ 30%	>40%
Saturated fat (% of energy intake)	≤ 10%	>13%
Dietary cholesterol	≤ 300 mg	>400 mg
% Recommended servings of fruit per day (2−4 based on energy intake)	≥ 100%	<50%
% Recommended servings of vegetables/day (3−5 based on energy intake)	≥ 100%	<50%
% Recommended servings of bread per day (6−11 based on energy intake)	≥ 100%	<50%
Calcium (% AI for age)	≥ 100%	<50%
Iron intake (% 1989 RDA for age)	≥ 100%	<50%
Dietary diversity score	≥ 6	<3
Dietary moderation score	≥ 7	<4

[a]*Scoring range for each component is 0 (minimum) to 10 (maximum).*
Source: Data from P.S. Haines, A.M. Siega-Riz, B.M. Popkin, The Diet Quality Index Revised: a measurement instrument for populations. Copyright © The American Dietetic Association. Reprinted by permission from J. Am. Diet. Assoc., 99 (1999) 697−704.

cholesterol consumed; recommended servings for fruit, vegetables, and grains; adequacy of calcium and iron intake; dietary diversity; and dietary moderation. The dietary diversity score was developed to show differences in intake across 23 broad food group categories, including 7 grain-based products, 7 vegetable components, 2 fruit and juice categories, and 7 animal-based products [40]. Dietary moderation scores added sugars, discretionary fat, sodium intake, and alcohol intake. The DQI-R was designed to monitor dietary changes in populations but can provide an estimate of diet quality for an individual relative to the national guidelines and can note improvement or decline of diet quality with multiple calculations [40].

The HEI [39,48] was first developed in 1995 by the USDA Center for Nutrition Policy and Promotion to assess and monitor the dietary status of Americans [48]. It has undergone several revisions to use a density-based scoring metric and reflect updated Dietary Guidelines for Americans, including a revision known as HEI-2010 [39]. The HEI-2010 captures the key recommendations of the 2010 Dietary Guidelines and, like earlier versions, is used to assess the diet quality of the US population and subpopulations, in evaluating interventions, in dietary patterns research, and to evaluate various aspects of the food environment. The HEI-2010 contains 12 components, including 9 adequacy and 3 moderation components; it uses a density approach to set standards expressed as a percent of calories or per 1000 calories; and it employs least restrictive standards, i.e., those that are the easiest to achieve among recommendations that vary by energy level, sex, and/or age (Table 8.2) [48].

The Dietary Patterns Methods Project (DPMP) was initiated in 2012 to strengthen the research evidence on dietary indices and dietary patterns [49]. The HEI-2010, Alternative HEI-2010 (AHEI-2010), aMED, and DASH scores showed a high degree of correlation and consistent

TABLE 8.2 Healthy Eating Index, 2010—Components and Standards for Scoring[a]

Component	Maximum Points	Standard for Maximum Score	Standard for Minimum Score (0)
Total fruit[b] (includes 100% juice)	5	≥0.8 cup equiv. per 1000 kcal	No fruit
Whole fruit[c] (includes all forms except juice)	5	≥0.4 cup equiv. per 1000 kcal	No whole fruit
Total vegetables[d]	5	≥1.1 cup per 1000 kcal	No vegetables
Greens and beans[d]	5	≥0.2 cup equiv. per 1000 kcal	No dark green vegetables or beans and peas
Whole grains	10	≥1.5 oz. equiv. per 1000 kcal	No whole grains
Dairy[e]	10	≥1.3 cup equiv. per 1000 kcal	No dairy
Total protein foods[f]	5	≥2.5 oz. equiv. per 1000 kcal	No protein foods
Seafood and plant proteins[f,g]	5	≥0.8 oz. equiv. per 1000 kcal	No seafood or plant proteins
Fatty acids[h]	10	(PUFAs + MUFAs)/SFAs > 2.5	(PUFAs + MUFAs)/SFAs < 1.2
Moderation			
Refined grains		<1.8 oz. equiv. per 1000 kcal	4.3 oz equiv. per 1000 kcal
Sodium	10	≤1.1 g per 1000 kcal	≥2.0 g per 1000 kcal
Empty calories[i]	20	≤19% of energy	≥50% of energy

[a] *Intakes between the minimum and maximum standards are scored proportionately.*
[b] *Includes fruit juice.*
[c] *Includes all forms except juice.*
[d] *Includes any beans and peas (called legumes in HEI-2005) not counted as total protein foods (called meat and beans in HEI 2005).*
[e] *Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.*
[f] *Beans and peas are included here (and not with vegetables) when the total protein foods (called meat and beans in HEI-2005) standard is otherwise not met.*
[g] *Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as total protein foods.*
[h] *Ratio of poly- and monounsaturated fatty acids to saturated fatty acids.*
[i] *Calories from solid fats, alcohol, and added sugars; threshold for counting alcohol is >13 g/1000 kcal.*
Source: Taken from http://www.cnpp.usda.gov/healthyeatingindex (accessed 15.03.16).

classifications among participants in three large, diverse cohorts (the AARP Diet and Health Study, the Multiethnic Cohort, and the Women's Health Initiative Observational Study—WHI-OS). It is encouraging that the DPMP researchers concluded that all four indices captured the essential components of a healthy diet [49].

Categorizing foods into appropriate groups, particularly combination foods, can be a challenge when using these analysis techniques. Guidelines are available to help overcome two major obstacles when assessing food intake with respect to the dietary guidelines—which are dealing with food mixtures and the differing units of measurement used. Because many foods are eaten as mixtures and are difficult to categorize into food groups, Cleveland et al. [50] developed a recipe file that helps break down food mixtures into ingredients so they can be assigned to their respective groups more easily. Standard serving sizes were assigned gram weights to help to overcome the unit problem, allowing for the use of only one unit of measure. The USDA Food Patterns Equivalents Database allows researchers to easily disaggregate combination foods into simple food group components using standardized cup- and ounce-equivalent units.

III PRESENTATION OF DATA

The presentation of data depends on the research questions for each study. For example, for a cross-sectional dietary assessment of an ethnic population, it may be useful to compare the data against population standards. These standards may include the DRI or comparison to a national average, such as the National Health and Nutrition Examination Survey (NHANES). Batis et al. [51] demonstrate how dietary differences among ethnic populations may be presented. Researchers may also wish to evaluate menus in comparison to dietary intakes. In a study involving 40 New York child care centers, researchers compared the dietary intakes of children to the dietary recommendations and found that less than 50% of children ate at least half of the daily recommended intake for each of the five food groups [52]. When comparing data for analyses, researchers must take into account differences that may exist among survey methods, questionnaire wording, data processing, and databases that could impact comparisons.

The DRIs are a set of nutrient-based reference values that include an estimated average requirement (EAR), an RDA, and an adequate intake (AI), which are defined by nutrient adequacy and may relate to the reduction of the risk of chronic disease [53]. Once the EARs have been established, they are used to set the RDAs, which should be used as a daily intake goal by healthy individuals and should be sufficient to meet the needs of 97% or 98% of all healthy people. If there is not sufficient evidence to determine an EAR, then an AI is set, once again based on groups of healthy people. A tolerable upper intake level (UL) is set when information is available as an indicator of excess for nutrients [53]. Each value has a specific goal and use [53] (Table 8.3). For example, the EAR is the estimate that is believed to meet the nutrient needs of half of the healthy people in a gender or life-stage group. When assessing nutrient intake of healthy groups, the EAR should be used instead of the RDA [53].

DRIs are set for specific subgroups based on age and sex. They are to be applied to healthy populations and may not be adequate for those who are or have been malnourished or have certain diseases or conditions that increase nutrient requirements. For individuals, the RDA and AI can serve as a goal for nutrient intake. A more complete description of the DRIs can be found in a later chapter.

TABLE 8.3 Uses of DRIs for Healthy Individuals and Groups

Type of Use	For the Individual	For a Group
Planning	RDA: Aim for this intake.	EAR: Use in conjunction with a measure of variability of the group's mean intake of a specific population.
AI: Aim for this intake.		
UL: Use as a guide to limit intake; chronic intake of higher amounts may increase risk of adverse effects.		
Assessment[a]	EAR: Use to examine the possibility of inadequacy; evaluation of true status requires clinical, biochemical, or anthropometric data.	EAR: Use in assessment of the prevalence of inadequate intakes within a group.

[a]*Requires statistically valid approximation of usual intake.*
AI, adequate intake; *EAR*, estimated average requirement; *RDA*, recommended dietary allowance; *UL*, tolerable upper intake level.
Source: Reprinted with permission from the National Academies Press. Copyright © 2005, National Academy of Sciences, Institute of Medicine and the National Academies (2005). Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, National Academy Press, Washington, DC.

TABLE 8.4 Overview of WWEIA

Five-step USDA AMPM used for collecting interviewer-administered 24-hour dietary recalls	Day 1: Day 1 in-person at the Mobile Exam Center
Day 2: Day 2 from central NHANES telephone center	
For each food and beverage, including water, consumed by a survey participant	• Name, identified by a USDA food code and description • Amount consumed, in grams • Amounts of food energy and 64 nutrients/food components provided by each food/beverage • Identification of items eaten in combination (e.g., cereal with milk added) • Separate ingredients coded for many salads and sandwiches • Day of week • Eating occasion—name (breakfast, lunch, etc.) • Time when each item was consumed • Source of food/beverage (where obtained) • Whether the food/beverage was eaten at home or not
For each survey participant	• Daily aggregates of energy and 64 nutrients/food components • Whether the day's intake was usual, much more than usual, or much less than usual • Salt type and use in food preparation and at the table • Whether on a special diet and type of diet • Frequency of fish/shellfish consumption in past 30 days (participants' age 1 year or older)

Some researchers use national survey data as a standard when presenting dietary data. WWEIA is the dietary intake component of NHANES. This survey is a joint effort between the USDA and the US Department of Health and Human Services, with data released in 2-yearly intervals. NHANES provides medical history, physical measurements, biochemical evaluation, physical signs and symptoms, and diet information from two 24-hour recalls using USDA's Automated Multiple-Pass Method (AMPM) [54]. Table 8.4 provides an overview of the dietary information collected. Researchers may wish to compare results of the information obtained from these surveys to determine how their study sample compares to the national average. Although the data from these surveys may be applied to certain subgroups, such as specific age groups, sex, socioeconomic levels, education levels, and some ethnic groups, they cannot be used as guides for others, such as malnourished or specific disease states.

IV INTERPRETATION OF DATA

Once the dietary intake data have been checked for errors, analyzed, and compared to a standard, researchers must then examine the results to determine what the data really mean. How the data are interpreted can depend on the research questions, the dietary assessment method used and the nutrient being studied, the study type, and the accuracy of participants' responses.

A Assessment Methods

The assessment method chosen for use in a study can determine how the data collected can be interpreted. Recalls and records gather present intake data, whereas FFQs provide data based on past intake. It is known that a person's nutrient and energy intake varies not only from day to day but also from season to season. Thus, if past intake is needed, FFQs may be the better choice.

The number of days of food intake records or recalls available can also affect the interpretation of the data. If high levels of accuracy are needed for a person's nutrient intake, a greater number of days will be required than if a group average could be used. Care must be taken when determining the number of days to use in a study. For example, researchers using data from a single 24-hour recall from 832 men found that saturated fat intake was inversely associated with stroke [55]. Because of the day-to-day variability, dietary changes or recommendations for an individual should not be based on a single day's intake. Basiotis et al. [56] determined the number of days of food intake data needed to estimate individual intake as well as group intake for food energy and 18 nutrients. They found that for females, an average of 35 days were needed to determine a true average of energy intake for each women, whereas 3 days of food records from each subject were required to determine a group average. A minimum of 6 days of food records were needed to

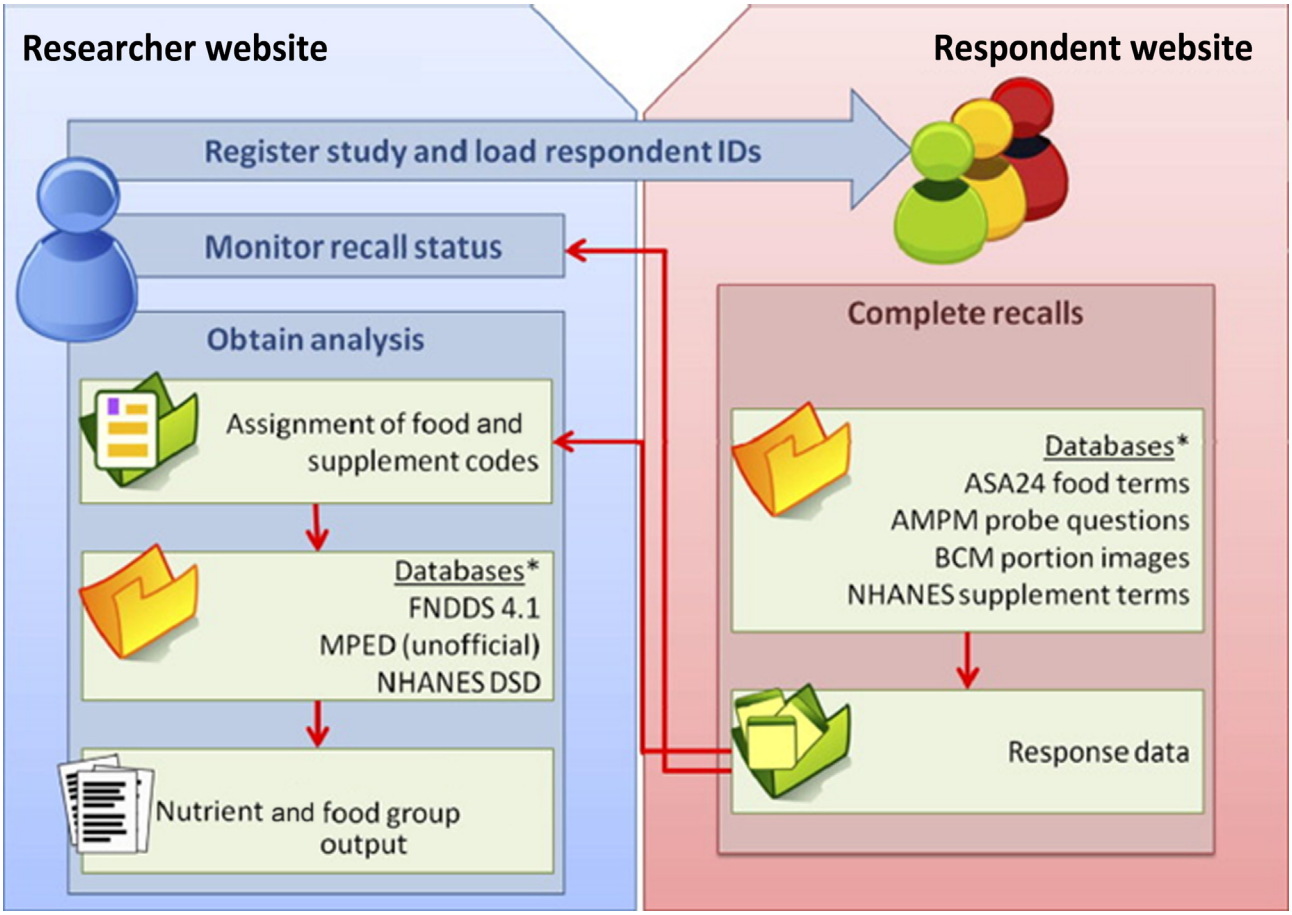

FIGURE 8.1 ASA24— *Taken from J. Acad. Nutr. Diet. 112(8) (2012) 1134–1137, doi:10.1016/j.jand.2012.04.016. Copyright © 2012 Academy of Nutrition and Dietetics.*

assess vitamin K in an elderly population [57]. However, participant burden also needs to be considered because longer recording periods may result in lower quality records as the number of days of recording increases [58].

It is also important that data obtained from dietary assessment methods be used properly. The FFQ was developed to rank nutrient intakes from low to high. It was not intended to be used to determine and develop levels of nutrient intake to prevent disease. However, many researchers have chosen to use the FFQ for this purpose. For example, studies based on FFQ data have recommended levels of vitamin E intake to reduce risk for heart disease [59,60]. However, other studies have concluded that these levels may not have the effect on the development of heart disease that was previously suggested [61–63]. When choosing a method for dietary assessment, care must be taken to ensure that the data are properly interpreted.

The Automated Self-Administered 24-hour Recall (ASA24) is a Web-based tool that was developed by researchers with the National Institutes of Health/National Cancer Institute to improve the feasibility of collecting high-quality dietary intake data from large samples [64]. See Fig. 8.1. Research demonstrates that the ASA24 performs well and offers substantial cost savings over interview-administered recall methods. Detailed information about the ASA24 including instructions to register and to use it free of charge is available at http://epi.grants.cancer.gov/asa24/.

B Data Validity

One major concern when interpreting dietary data is the accuracy of the information reported. Because most of the information gathered is self-reported, the reliability and validity of the data depend on the reporter's motivation, memory, ability to communicate, and awareness of the foods consumed. Most methods have been proven to be generally reliable; i.e., they will provide the same estimate on different occasions. However, do the techniques gather

true and accurate measurements, or valid measurements, of what people are really eating? In the past, assumptions were made that the information was indeed valid. The validity of the techniques was often verified by comparing the different methods to each other. This technique has been referred to as calibration [65]. For example, the results of an FFQ are compared with results obtained using a food record or repeated 24-hour recalls. The estimates by the different methods will differ for a number of reasons. The FFQs rely on the participant's memory to recall foods eaten over a specified period of time. Some participants may have difficulty in remembering this level of detail. Alternatively, food records rely on participants' willingness to record in great detail all food items and beverages consumed. The burden of this task is known to lead to participants changing their intake. It is important to be aware that all self-report methods will have measurement error. This presents a major issue for data interpretation because imprecise methods may obscure the diet–disease relationship. This issue is discussed in detail by Freedman and colleagues [66]. A way to address measurement error in dietary assessment methods is by use of external recovery biomarkers [65,67]. Doubly labeled water (DLW) is the most widely used biomarker for energy intake. Other biomarkers that have been used to validate nutrient intake include fatty acid patterns in blood to reflect fatty acid intake [68], urinary nitrogen to validate protein intake [69,70], serum carotenoids and vitamin C concentrations as markers of fruit and vegetable consumption [71,72], as well as urinary sucrose and fructose as a marker of total sugar intakes [73]. With the increased use of these biomarkers, particularly DLW, to determine the accuracy of dietary intake data in a variety of subjects, reporting errors will be better defined. The use of dietary biomarkers may even help to compensate for reporting errors related to some nutrients—e.g., urinary sucrose and fructose as a biomarker for total sugar intakes. Misreporting of food intake—over- or underreporting—occurs with all self-report methods of dietary assessment [67].

A concerted effort is being made to develop and identify objective dietary biomarkers to aid in defining dietary exposures. Dietary biomarkers are objective biochemical indicators of dietary intake or nutritional status that accommodate the error in self-report methods and thus shed light on the diet–disease relationship [74]. Biomarkers may also be defined as a biochemical indicator of dietary intake/nutritional status, an index of nutrient metabolism, or a marker of the biological consequences of dietary intake [75]. There are three classes of nutritional biomarkers: recovery, concentration/replacement, and predictive biomarkers [75]. In order to obtain independent observations, epidemiologists have utilized concentration biomarkers assessed in biological samples as predictors of disease risk. High-density lipoprotein cholesterol and low-density lipoprotein cholesterol are examples of well-known biomarkers that are strongly associated with risk for cardiovascular disease. One of the main uses of dietary biomarkers is as a reference measurement to assess the validity and accuracy of dietary assessment methods. Recovery biomarkers are based on the concept of metabolic balance between intake and excretion over a fixed period of time [75]. Recovery biomarkers such as DLW and urinary nitrogen help to quantify errors in self-report of energy and protein intake, respectively. The concept of predictive biomarkers is relatively new. These biomarkers are sensitive, time dependent, and show a dose–response relationship with intake levels [73]. Although the correlation to intake is high, the actual recovery of the marker is quite low. Urinary fructose and sucrose concentrations represent predictive biomarkers that have been shown to significantly correlate with sugar intake and provide a useful, independent qualitative index of sugar consumption [73]. Urinary sucrose and fructose have been used in the Observing Protein and Energy Nutrition (OPEN) study to assess for error in self-reported intake of total sugars [76]. Researchers propose that predictive biomarkers, like recovery biomarkers, may be useful for validation and calibration studies to estimate the error in self-reported dietary intakes. Predictive biomarkers may also be used independently of self-report to categorize people by their level of nutrient intake. Inherent errors (e.g., underreporting and undereating) in self-report of dietary intake may be difficult to eliminate. However, the use of dietary biomarkers will accommodate for the errors in self-report methods and thus shed light on the diet–disease relationship. For the researcher, the issue is how best to deal with implausible records as well as continuing to try to identify those participants most likely to overreport and why.

1 Overreporting

Overreporting occurs when reported intakes are higher than the measured energy expenditure levels. Overreporting has not been found to be as large of a problem with regard to reported energy intakes as underreporting, but it still has the potential to interfere with results and conclusions. This occurs particularly if the overreported foods are low in energy but high in nutrients such as many vegetables. Johansson et al. [77] found that people who overreport tend to be younger and, with lower body mass indexes (BMIs), and are often considered lean. The highest proportion of overreporters was found among those subjects who wanted to increase their body weight. A study of the elderly population found that overreporting was higher in men [78]. Although overreporters are not common, care should be taken when obtaining data from participants who have characteristics related to risk of overreporting.

2 Underreporting

Underreporting occurs when reported intakes are lower than measured energy expenditure levels or estimated energy requirements. These reports are often so low that basal metabolic needs could not be met, and they are not biologically plausible. Depending on the age, gender, and body composition of a given sample, underreporters may comprise 2–85% of the total sample [79]. It is now understood that underreporting tends to be associated with certain groups. Prevalence of underreporting also increases as BMI increases. Many studies have shown that the obese underreport more often and to a greater degree (30–47%) than the lean [80–84]. Women have also been found to underreport more often than men [82,85–90]. The EPIC 24-hour recall data indicated that BMI and age were consistently related to underreporting among 35,955 men and women aged 35–74 years [90]. The researchers note, however, that the association observed may simply be due to the common source of variability between EI (energy intake)/basal metabolic rate (BMR) and its components and not to a true causal relationship between BMI, age, and underreporting. Table 8.5 provides a summary of groups most likely to be underreporters. These factors are further complicated by the possibility that they are risk factors for many chronic diseases.

TABLE 8.5 Summary of Underreporting

Populations Most Likely to Underreport	
Women [77,90]	Smokers [77,86]
Higher BMI [77,83,84,89–91]	Lower education [82]
Low socioeconomic status, lower income [83,86,89]	Ethnicity [83]
Psychosocial Characteristics Associated With Underreporting	
High scorers on restrained eating scales [92]	A history of dieting behavior [84,92]
Body dissatisfaction [89,93]	Social desirability [84,89]
Fear of negative evaluation [84]	
Foods Most Likely to Be Underreported	
US survey [94]	
Cake/pie	Meat, fish, poultry, egg sandwiches, or mixtures
Savory snacks: chips, popcorn, pretzels	Regular soft drinks
Cheese	Fat-type spreads
White potatoes	Condiments
British survey [93]	
Cake	Breakfast cereal
Sugars	Milk
Fats	

3 The Problem with Underreporting

A major problem with underreporting occurs when researchers begin to classify dietary intake information to determine diet and disease associations. This is often done by ranking nutrient intakes from low to high and then searching for any associations between nutrient intake and the occurrence of disease. There is a danger of misclassification of subjects if this ranking is based on false or underreported intakes. As noted previously, those who tend to be at higher risk for underreporting are also those who are at greater risk for many chronic diseases. For example, obesity is a known risk factor for coronary heart disease as well as underreporting. Because bias in measuring dietary intake has the potential of removing as well as creating associations, it can generate misleading conclusions about the impact of diet and disease [73,74]. Underreporting is a potentially misleading problem in nutrition research. The use of biomarkers can help to validate and objectively interpret dietary intake data and, ideally, should become routine in nutritional epidemiology. However, the cost of some biomarkers such as DLW may make it impractical for routine use for researchers.

4 Reasons for Underreporting

With the aid of biomarkers for dietary intake, reporting error has been defined. Researchers continue to search for reasons why people underreport. We know that being obese is not the cause of underreporting, but it is most likely the psychological and behavioral characteristics associated with obesity that lead to underreporting [79,95]. Social desirability and self-monitoring are contributors to reporting errors [84,92]. A need for social acceptance, a desire to be liked or accepted by the interviewer, may cause the subject to underreport "sinful" foods or report "healthy" foods that were not actually consumed. A high level of body dissatisfaction—i.e., if a person sees a leaner physique as being healthier or more desirable than his or her own—may cause the person to misreport foods [93]. Also, researchers found that women who scored higher on restrained eating scales, those who believe they are making a conscious effort to avoid certain foods, tend to underreport as well [79,92,95]. For some people, the act of recording dietary intake may lead to changes in eating behaviors. Thus, a person may be reporting actual, but not usual, intakes.

Another explanation for underreporting may be related more to meal size than body size. Wansink and Chandon [96] asked overweight and normal-weight adults to estimate the calories in both small and large fast-food meals.

They found that both groups underestimated the number of calories in the larger meals. Therefore, greater underestimation of energy by obese individuals may be, in part, a result of their tendency to consume larger meals.

5 Foods Most Often Underreported

Underreporting does not occur for all foods and nutrients to the same degree. Foods that are perceived as unhealthy are reported less frequently and in smaller portions among low energy reporters. In a US survey of 8334 adults, 1224 were found to be low energy reporters [94]. Foods that were found to be most often underreported included cakes/pies, savory snacks, cheese, white potatoes, meats, regular soft drinks, fat-type spreads, and condiments [94]. British researchers found little difference between underreporters and plausible energy reporters with regard to bread, potatoes, meat, vegetables, or fruit, but a significant difference was seen with cakes, sugars, fat, and breakfast cereal [97]. Participants completing a 24-hour dietary recall after being observed for 24 hours in a metabolic unit frequently did not report between-meal eating events [98]. This may further contribute to the most often underreported foods. Table 8.5 gives a summary of underreported foods. Some researchers have found that underreporters tend to report lower intakes of fat and higher intakes of protein and carbohydrates as a percentage of total energy [98,99], whereas others show that reports of added sugars intake are significantly lower [98]. The OPEN study used DLW to identify low energy reporters [100] and found they were more likely to report smaller portions, and female low energy reporters reported less soft drink consumption. No agreement has been reached as to how much, if at all, specific macronutrients are misreported.

TABLE 8.6 Principles of the Goldberg Cutoff

Principle	Equations and Comments
Principle 1: Validation of reported energy intake rests on the following:	Energy intake (EI) = energy expenditure (EE) − changes in body stores
Principle 2: Assumes that subjects are weight stable and therefore are in energy balance:	EI = EE
Principle 3: Express energy requirements as multiples of BMR.	EE = physical activity level (PAL) × BMR
Principle 4: Since EI = EE and EE = PAL × BMR, then the following can be assumed:	EI = PAL × BMR or EI: BMR = PAL
Principle 5: Reported energy intake expressed as:	EI_{rep}: BMR can be compared with expected PAL
Principle 6: Since error exists in all the measured elements of the equation, absolute agreement cannot be expected.	
Principle 7: Confidence limits (cutoffs) of the agreement between:	EI_{rep}: BMR and PAL must be determined to establish if reported values within the subjects are acceptable. (For cutoff equations, see Black et al. [93].)

Source: Data from A.E. Black, Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate: a practical guide to its calculation, use and limitations. Int. J. Obes. Relat. Metab. Disord. 24 (2000) 1119–1130.

6 Identifying Underreporters

To help identify underreporters, researchers can apply methods such as the Goldberg cutoff, extensively described by Goldberg and colleagues [101] and Black and colleagues [102,103]. The Goldberg cutoff identifies the most obvious implausible intake values by evaluating the energy intake against estimated energy requirements. BMR can be measured using methods described elsewhere, or height and weight measurements can be used to predict BMR from a standard formula (the Schofield equation is recommended by Goldberg et al. [101]). A ratio of the estimated EI to measured or predicted BMR is calculated as EI/BMR. This ratio can then be compared with a study-specific cutoff value (see Black [102] for a practical guide to using the Goldberg cutoff). This cutoff represents the lowest value of EI/BMR that could reasonably reflect the energy expenditure based on the physical activity level if the information is available or assuming a sedentary lifestyle if no activity information is gathered. A summary of the principles of the cutoffs can be found in Table 8.6. Studies using the Goldberg cutoff classified 28–39% of the women and 18–27% of the men as low energy reporters [86,94].

The Goldberg cutoff has several limitations [102]. It has poor sensitivity for defining invalid reports at the individual level because it identifies only the extreme underreporters. It also does not distinguish between varying degrees of underreporting. The major limitation is that the cutoff depends on knowledge of energy requirements or energy expenditure. If no physical activity information is available, the cutoff assumes a sedentary lifestyle and will therefore underestimate the underreporters and may lead to misclassification of energy reporting. If researchers can gather information on lifestyle, occupation, leisure time activities, and particularly information regarding physical activity of the participants, calculations can be more specific and improve the classification of energy reporting (i.e., plausible, underreporting, or overreporting).

To compensate for the physical activity level limitations, McCrory et al. [104,105] proposed a method based on the

principles of the Goldberg cutoff to screen for implausible reports by comparing reported energy intake with predicted total energy expenditure [104,106]. Researchers offer that because total energy expenditures are predicted, not just BMR, this new method eliminates the potential error caused by assigning inaccurate physical activity levels when there is limited or no information on activity levels available [104]. With the Goldberg method, only those participants who report ± 2 standard deviation cutoffs are considered underreporters. However, McCrory et al. [104] suggest that in some studies, particularly those in which relationships between habitual intakes and disease outcomes are examined, using a 1 standard deviation cutoff may help to identify those reporters who report actual but not habitual diet. Using this method to analyze CSFII 1994–1996 data, Huang et al. [104] found that implausible reported energy intakes reduced the overall validity of the sample and including misreporters could lead to inappropriate conclusions about the impact of diet on health outcomes. Thus, in the absence of objective measures of total energy expenditure (TEE) or physical activity, the Goldberg method is a reasonable approach to characterize underreporting [107].

7 Handling Underreporting in Dietary Data

Researchers are still not sure how to handle data sets containing large numbers of underreporters. Several approaches have been suggested, but none are ideal. One technique is to exclude anyone who is found to report implausible energy intakes. The problem with this method is that the underreporters tend to fall into specific subgroups (i.e., obese and smokers) and, as stated previously, eliminating them will alter the sample. Some investigators have analyzed their data with all the subjects and then again after the underreporters were removed [108]. Among adults from Pacific Northwest tribal nations, plausible reporters completing dietary records were found to have higher estimates of vitamins A, C, and E, magnesium, and sodium [109]. For the plausible reporters in this population, the association between reported energy intakes (from dietary records and from FFQs) and weight was significant [110]. However, when the sample was not limited to plausible reporters, the association between reported energy intakes and weight was not significant.

Other researchers have suggested adjusting nutrient intakes for energy intake using the regression of nutrient versus energy [111]. This would be feasible only if portion sizes were underestimated but the actual foods were all reported accurately. Otherwise, this method could make the reports worse [112]. As noted previously, it is most likely that foods are systematically omitted from recalls. Thus, if, e.g., fat-containing foods (e.g., desserts) are often underreported, whereas vitamin A-containing foods (e.g., cantaloupe) are not, energy adjustments would provide lower than actual measures of fat intake but a higher measure of vitamin A intake. Many researchers have recognized that adjustments cannot eliminate the bias caused by selective underreporting [113].

Recently authors claimed that, because of energy intake underreporting, measurement error from self-reported dietary intake data is so great that findings that rely on them are of no value [114]. In response, a writing group of esteemed nutritional epidemiologists countered that the amassed evidence shows the self-reported dietary intake data can successfully be used to inform dietary guidance and public health policy [115]. These scientists published seven specific recommendations for collecting, analyzing, and interpreting self-reported dietary intake data [115]. See Table 8.7.

8 Improving Dietary Assessment with Technology

Variations on the 24-hour dietary recall, food records, and FFQs have been carefully developed and improved

TABLE 8.7 Recommendations for Collecting, Analyzing, and Interpreting Self-Reported Dietary Intake Data

1. Continue to collect self-report dietary intake data because they contain valuable, rich, and critical information about foods and beverages consumed by populations that can be used to inform nutrition policy and assess diet–disease associations.
2. Do not use self-reported energy intake as a measure of true energy intake.
3. Do use self-reported energy intake for energy adjustment of other self-reported dietary constituents to improve risk estimation in studies of diet–health associations.
4. Acknowledge the limitations of self-report dietary data and analyze and interpret them appropriately.
5. Design studies and conduct analyses that allow adjustment for measurement error.
6. Design new epidemiologic studies to collect dietary data from both short-term (recalls or food records) and long-term (FFQs) instruments on the entire study population to allow for maximizing the strengths of each instrument.
7. Continue to develop, evaluate, and further expand methods of dietary assessment, including dietary biomarkers and methods using new technologies.

Source: Data from A.F. Subar, L.S. Freedman, J.A. Tooze, S.I. Kirkpatrick, C. Boushey, M.L. Neuhouser, et al., Addressing current criticism regarding the value of self-report dietary data, J. Nutr. 145 (12) (2015) 2639–2645, doi:10.3945/jn.115.219634.

upon throughout the years; however, the errors discussed previously still remain. Technological advances have prompted researchers to investigate the use of technology to improve diet assessment methods. Efforts to use available technology include applications for personal computers and mobile devices, as well as the development of objective biomarkers for dietary intakes [116]. Technology-based dietary assessment holds promise for engaging participants and reducing participant burden, as well as alleviating many researcher burdens (e.g., data entry and interview staff), thus improving cooperation and accuracy [116,117]. Objective biomarkers hold promise for improving associations between dietary intakes and disease outcomes in tandem with or independent of dietary assessment methods.

As technology has rapidly evolved and become more widely adopted by the general public, applications for mobile devices with integrated digital cameras have become desirable tools for use in the research community. Applications in which a participant or "user" takes images of foods and beverages consumed have become of increasing interest. Mobile applications are also viewed favorably by adolescent and adult study participants who report a preference for technology-based dietary assessment over pen-and-paper methods [1,118]. Proposed image-based dietary assessment methods can be categorized as follows: Images are reviewed by the participant/user, images are reviewed by a trained analyst, or automated systems review the images. One proposed use of digital images is to supplement a 24-hour dietary recall—in other words, an image-assisted dietary recall. Arab and Winter [119] proposed a method in which a user wears a mobile device that hangs around his or her neck for a 24-hour period. During this time, intermittent images are automatically taken. The images containing food and beverages are presented to the user to help him or her remember all foods and beverages during the 24-hour dietary recall [119]. In another study the use of wearable cameras by participants reduced the magnitude of underreporting by 8% for men and 7% for women compared with the 24 hour recall alone [120]. Although the digital images of foods reviewed by the user reduce the memory-related burden, issues related to portion size estimation still exist. Applications in which images taken by a participant are reviewed by a trained analyst to remove the portion size estimation burden from the user. In one example, the remote food photography method, analyst estimates range from approximately −5% to 7% of actual energy intake [118]. Another study used digital imaging to measure elementary school children's fruit and vegetable intake. The researchers found that mean fruit and vegetable consumption using digital imaging was not significantly different from the gold standard of weighed plate waste and was within 1.0 g of weighed plate waste (about the weight of one pea pod) [121].

Although accuracy of energy intakes would be improved with this method, there remains a burden on research staff to visually analyze the images. To further remove burden from the user as well as the researcher in an effort to improve the accuracy of dietary intakes, nutrition researchers are teaming with electrical engineers to develop an application in which the images are primarily analyzed using an automated system [122−124]. The Technology Assisted Dietary Assessment (TADA) mobile telephone food record (mpFR) application has been developed for mobile devices with the goal of providing a more accurate method for dietary assessment. The researchers believe they have developed a tool that will be useful for replacing the traditional food record methods currently used and they continue to refine and develop the system to increase its accuracy and usability [124].

As technology continues to evolve, we can expect that image-based dietary assessment will become more mainstream as a means for ameliorating self-reporting errors currently seen in diet assessment methods.

CONCLUSION

Dietary assessment methods provide valuable data to measure dietary exposure in nutritional epidemiology. When undertaking dietary assessment, attention should be given to ways in which error can be minimized throughout the data gathering and analysis process. Identifying underreporters and improving dietary database validity through analytical approaches should remain in the forefront of dietary assessment until methodology improvements can be found. Improving dietary intake methodology is critical to the credibility of nutrition research, and improvements in technology may be a way forward to achieving this goal.

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Chapter 9

Current Theoretical Bases for Nutrition Intervention and Their Uses

Yaguang Zheng[1], Juliet Mancino[2], Lora E. Burke[3] and Karen Glanz[4]

[1]*Boston College Connell School of Nursing, Chestnut Hill, MA, United States,* [2]*University of Pittsburgh School of Medicine, Pittsburgh, PA, United States,* [3]*University of Pittsburgh School of Nursing, Pittsburgh, PA, United States,* [4]*University of Pennsylvania, Philadelphia, PA, United States*

I INTRODUCTION

Dietary interventions are a central component of disease prevention and management. Health professionals' roles in dietary interventions are pivotal because of their centrality in health care and their credibility as patient educators [1−4]. The most recently published evidence-based recommendations in the United States advice to include intensive behavioral dietary counseling for adults with known risk factors for chronic disease, noting that this counseling can be provided by physicians or other clinicians such as dietitians [5,6]. Although a recent report from the US Preventive Services Task Force stated that there is inadequate direct evidence that intensive behavioral counseling interventions lead to decreases in cardiovascular morbidity or mortality in adults with cardiovascular disease risk factors [5], earlier reports noted that people who report receiving advice or counseling recommending dietary change report more health-enhancing diet changes and weight loss than do those who received no such advice [7,8]. Furthermore, nonclinical community sites such as worksites, churches, schools, and community centers are becoming increasingly important as settings for nutritional information and dietary interventions, especially with the epidemic rates of obesity and diabetes among US children, adolescents, and adults [9−11].

II THE IMPORTANCE OF UNDERSTANDING INFLUENCES ON DIETARY BEHAVIOR

Successful dietary interventions take many forms. Interventions to yield desirable changes in eating patterns can be best designed with an understanding of relevant theories of diet-related behavior change and the ability to use them skillfully [12]. Although earlier reports of dietary interventions did not cite a particular theory or model as the basis for the strategies they employed [13,14], the application of sound behavioral science theory in dietary interventions has become more the norm today [15]. Also, emerging evidence suggests that interventions developed with an explicit theoretical foundation are more effective than those lacking a theoretical base [16,17] and that combining multiple theories may lead to better outcomes (e.g., social cognitive theory (SCT) and the transtheoretical model (TTM)) [18,19]. In recent years, empirical evidence demonstrates that mobile health (mHealth) interventions can achieve significant improvement in dietary intake [20]; in particular, the studies using health behavior theories to guide mHealth interventions showed improvements of behavior changes in dietary intake [21,22].

Six theoretical models have been used often in studies targeting dietary change and thus have been demonstrated to be useful for understanding the processes of changing eating habits in clinical and community settings as well as guiding the development of dietary interventions: SCT, which includes self-efficacy and self-regulation—constructs that have been used extensively in diet and weight loss studies; the stages of change construct from the TTM; consumer information processing; the theory of planned behavior (TPB); multiattribute utility theory (MAU); and the social ecological model [12,23,24]. This chapter describes the central elements of each theory and how the theories can be used to guide the development of interventions and provide a framework for the interpretation of outcomes.

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A Multiple Determinants of Food Choice

Many social, cultural, and economic factors contribute to the development, maintenance, and change of dietary habits. No single factor or set of factors has been found to adequately account for why people eat as they do. Physiologic and psychological factors, acquired food preferences, and knowledge about foods are important individual determinants of food intake. Families, social relationships, socioeconomic status, culture, geography, and access to food are also important influences on food choices. A broad understanding of some of the key factors and models for understanding food choice can provide a foundation for well-informed clinical dietary interventions, help identify the most influential factors for a particular patient, and enable clinicians to focus on issues that are most salient for their patients.

B Multiple Levels of Influence

Common wisdom holds that nutrition interventions are most likely to be effective if they embrace an ecological perspective for health promotion [25,26]. That is, they should not only target individuals but also affect interpersonal, organizational, and environmental factors influencing diet-related behaviors [27,28]. This is most clearly illustrated when one thinks of the context of selecting and purchasing food. Consumers learn about foods through advertising and promotion via multimedia, labels on food packages, and through product information in grocery stores, cafeterias, and restaurants [29]. Their actual purchases are influenced by personal preferences, family habits, medical advice, availability, cost, packaging, placement, and intentional meal planning. The foods they consume may be further changed in the preparation process, either at home or while eating out. The process is complex and clearly determined not only by multiple factors but also by factors at multiple levels. Still, much food choice can be represented by routines and simple, internalized rules. Even though multilevel interventions proceed organically from socio-ecological theory by virtue of addressing various personal and environmental factors, actual multilevel interventions remain scarce [26].

Traditionally, health/patient educators focus on intraindividual factors such as a person's beliefs, knowledge, and skills. Contemporary thinking suggests that thinking beyond the individual to the social milieu and environment can enhance the chance of successful health promotion and patient education [25,30]. Health providers can and should work toward understanding the various levels of influence that affect the patient's behavior and health status. This is discussed and illustrated with examples later on in this chapter.

III WHAT IS THEORY?

A theory is a set of interrelated concepts, definitions, and propositions that present a systematic view of events or situations by specifying relations among variables in order to explain and predict the events or situations. The notion of generality, or broad application, is important [12]. Although various theoretical models of health behavior may reflect the same general ideas, each theory employs a unique vocabulary to articulate the specific factors considered to be important. Theories vary in the extent to which they have been conceptually developed and empirically tested.

Theory can be helpful during the various stages of planning, implementing, and evaluating interventions [12]. Theories can be used to guide the search for reasons why people are or are not consuming a healthful diet or adhering to a therapeutic dietary regimen. They can help pinpoint what you need to know before working effectively with a client, group, or patient. They also help to identify what should be monitored, measured, and/or compared in evaluating the efficacy of nutrition intervention.

IV EXPLANATORY AND CHANGE THEORIES

Theories can guide the search to understand why people do or do not follow health-related or therapeutic advice, help identify what information is needed to design an effective intervention strategy, and provide insight into how to design an educational program so it is successful [12]. Thus, theories and models help explain behaviors as well as suggest how to develop more effective ways to influence and change behaviors. These types of theory often have different emphases but are quite complementary [12]. For example, understanding why someone chooses the foods he or she eats is one step toward successful dietary management, but even the best explanations are insufficient by themselves to fully guide change to improve health. Some type of change model will also be needed. All of the theories and models described here have some potential as both explanatory and change models, although they might be better for one or the other purpose. For example, the TPB was originally developed as an explanatory model, whereas the stages of change construct was conceived to help guide planned change efforts.

V UNIQUE FEATURES OF DIET-RELATED BEHAVIOR TO CONSIDER WHEN USING THEORY

Diet-related behavior changes are most likely to be effective for preventing or managing disease when they are

sustained over the long-term and in people's natural environments, outside the clinical setting. To be effective in dietary interventions, health care providers need to understand both the principles of clinical dietary management and a variety of behavioral and educational issues [31].

There are several core issues about diet and eating-related changes that should be recognized. First, most diet-related risk factors are asymptomatic and do not present immediate or dramatic symptoms. Moreover, by the time symptoms are recognized, the changes needed are often very challenging—e.g., sodium restriction in the patient with congestive heart failure. Furthermore, health-enhancing dietary changes require qualitative change, not just modification of the amount of food consumed, and eating cessation is not a viable option (as with smoking or other addictive behaviors). Finally, both the act of making changes and self-monitoring require accurate knowledge about the nutrient composition of foods or a convenient, practical reference source, as well as a commitment to make change by the individual. Thus, information acquisition and processing may be more complex for dietary change than for changes in some other health behaviors, such as smoking and exercise. As such, consumer information processing models (described later) are more important for dietary interventions than for other types of health-related behaviors. Other important issues include long-term maintenance, the format and medium for providing dietary advice, nutritional adequacy, and options for initiating the change process, the ubiquitous availability of food, the ever-changing food supply, fad diets, and special populations.

A Long-Term Dietary Change

Because dietary intervention leads to meaningful improvements in health only when long-term change is achieved, both providers and patients need to "look down the road" when formulating expectations and setting goals [32]. For example, for most patients without other major risk factors or a familial disorder who follow recommended dietary changes for cholesterol reduction, significant reductions are seen within 4–6 weeks, and cholesterol reduction goals can be reached within 4–6 months [33]. Even after goals are achieved, new dietary habits must be maintained. Thus, if it takes several weeks or even months to adjust to the new dietary regimen, patience and persistence by both physician (dietitian, or clinician) and patient may be worthwhile in the long-term [34,35]. Maintenance of new eating habits occurs mainly outside any clinical or therapeutic setting. Also, different skills are required to make initial changes and to maintain them over the long-term, so follow-up consultations and advice should address new issues, not merely repeat or rehash old information. Reinforcement of the behavior changes that have occurred is paramount [36].

B Restrictive and Additive Recommendations: Typical Reactions

Traditionally, dietary intervention has focused on advice to restrict intake of certain foods or nutrients—e.g., reducing fat and saturated fat intake, limiting calorie intake, and limiting sodium/salt. Yet the most often mentioned obstacle to achieving a healthful diet is not wanting to give up the foods we like [37]. Basic psychological principles hold that when people are faced with a restriction or loss of a choice, that choice or commodity becomes more attractive. In other words, focusing mainly on what not to eat, or on eating less of some types of foods, may evoke conscious or unconscious negativism in some people. In contrast, counseling rather than advising the individual about making behavior changes and emphasizing additive recommendations, such as increasing intake of fruits and vegetables or eating more fiber-rich foods, often appeals to people because it sanctions their doing more of something and guides them in how to do it. The challenge is to make these recommendations attractive to individuals and to ensure that they are presented in the context of an overall healthful diet.

C Implications of Counseling for Gradual Change or Very Strict Diets

A generally held view is that the chances of long-term dietary change are greater when efforts to change occur in a gradual, stepwise manner and are viewed in the context of a lifestyle change rather than a diet. This might involve setting small goals for attempting changes within specific food groups one at a time, until the total diet comes close to recommendations. A basic principle involved is that small successes (i.e., recognition of each successful behavioral change) increase confidence and motivation for each successive change. Although this is effective for many people, others become impatient or even lose their enthusiasm for changes that are minimally recognizable. An alternative is to begin with a highly restrictive diet such as a very low-calorie weight loss diet, a very low-fat diet for prevention of cardiovascular disease, or a very low-sodium diet for an individual with kidney disease. These types of programs, with very strict dietary regimens, may be useful for patients who are highly motivated (e.g., postsurgically, after a coronary incident, or newly diagnosed) or for those who have not been successful in making gradual changes. In some cases, a strict diet for an initial short period will yield visible and/or clinical changes that help motivate patients to continue adhering

to a less extreme regimen; however, such diets may require careful supervision. Moreover, diets that require extreme calorie restriction for weight loss often result in a rapid weight regain after the diet is discontinued [34,35]. For these reasons, these markedly restricted diets are not recommended for most individuals.

D Special Populations

Ideally, each patient should be treated as an individual with unique circumstances and health history. Still, epidemiological research indicates that certain demographic subgroups differ in terms of both risk factors and diet. Understanding these population differences can help prepare a provider to work with various types of patients. Minority and lower socioeconomic status individuals are disproportionately affected by obesity [38] and related risk factors. Targeted interventions are needed for disadvantaged groups [39]. Age may also make a difference: younger persons may feel invulnerable to coronary events, and older adults may be managing multiple chronic conditions and using both prescribed and over-the-counter medications that could interact with foods. These are just a few examples of how population subgroups may differ, and they serve as a reminder to be sensitive to group patterns but to avoid stereotyping in the absence of firsthand evidence about an individual.

VI IMPORTANT THEORIES, THEIR KEY CONSTRUCTS, AND APPLICATION

Several available and widely used models and theories of behavior change can guide dietary interventions. This section describes the following six models and their constructs: SCT, including self-efficacy and self-regulation; stages of change from the TTM; consumer information processing; the TPB; multiattributed utility theory; and the social ecological model [12]. The central elements of each theory and how they can be used to help formulate and guide dietary interventions are described. Table 9.1 provides illustrative statements demonstrating the application of each theory. Following a review of theory constructs, we will discuss findings related to the application of each theory for dietary intervention.

A Social Cognitive Theory

SCT, the cognitive formulation of social learning theory that has been best articulated by Bandura [40,41], explains human behavior in terms of a three-way, dynamic, reciprocal model in which personal factors, environmental influences, and behavior continually interact. SCT synthesizes concepts and processes from cognitive, behavioral, and emotional models of behavior change, so it can be readily applied to dietary interventions that target behavior change for disease prevention and management. A basic premise

TABLE 9.1 Statements Representing Theoretical Approaches to Understanding and Changing Dietary Behavior

Theory	Statements
Social cognitive theory	Overeating at holidays is triggered by food advertisements, store displays, and party buffets that people encounter. Work with individual to set realistic, specific, and proximal goals; e.g., at holiday parties, have just one dessert.
Stages of change	The intervention should match the stage of readiness of the client. If someone believes that the time is right and is "ready to change," he or she will probably be more successful with a nutrition intervention. For the person who is in a contemplative stage, provide attractive literature that is appropriate for the individual to read to possibly motivate the person to move forward.
Consumer information processing	The information on nutrition labels sometimes "overloads" consumers. Consumers who are concerned about nutrition tend to look at nutrient labels before deciding which food to buy.
Theory of planned behavior	An individual who plans to change how he or she eats and specifies what, when, how, and where the changes will happen is more likely to follow through than someone with a more general plan. Furthermore, if the person thinks that his or her spouse will be supportive, motivation will be higher.
Multiattribute utility theory	If taste and convenience are foremost in someone's mind when deciding what to eat, a nutrition intervention that zeroes in on these factors has the greatest promise of success.
Social ecological model	If healthful food is easily available and low in cost, and there are good facilities for cooking and food preparation, individuals will be more likely to follow healthy eating patterns.

Source: Adapted in part from A. McAlister, C. Perry, G. Parcel, How individuals, environments, and health behaviors interact: social cognitive theory, in: K. Glanz, B.K. Rimer, K. Viswanath (Eds.), Health Behavior and Health Education: Theory, Research, and Practice, Jossey-Bass, San Francisco, CA, 2008, pp. 169–185 and J. Rudd, K. Glanz, How consumers use information for health action: consumer information processing, in: K. Glanz, F.M. Lewis, B.K. Rimer (Eds.), Health Behavior and Health Education: Theory, Research, and Practice, Jossey-Bass, San Francisco, CA, 1990, pp. 115–139.

of SCT is that people learn not only through their own experiences but also by observing the actions of others, particularly role models who seem credible, and the results of those actions [12]. Key constructs of SCT that are relevant to dietary intervention include observational learning, self-regulation [42], reinforcement, self-control, and self-efficacy [41,43].

Principles of behavior modification, which have often been used to promote dietary change, are derived from SCT. Elements of behavioral dietary interventions based on SCT constructs of self-control, self-regulation, reinforcement, and self-efficacy include goal-setting, self-monitoring, self-evaluation, feedback, and behavioral contracting [31,44]. As discussed later, goal-setting and self-monitoring are key behavioral strategies to support behavior change [45,46].

1 Self-Efficacy

Self-efficacy is a person's perception of how capable he or she is to perform a specific behavior or take action and persist in that action despite obstacles or challenges. Self-efficacy is behavior specific; e.g., a person may have high self-efficacy for initiating a physical activity program but low self-efficacy for changing dietary habits [41]. Health providers can make deliberate efforts to increase patients' self-efficacy using three types of strategies: (1) setting small, incremental, and achievable goals; (2) using formalized behavioral contracting to establish goals and specify rewards; and (3) monitoring and reinforcement, including having the patient self-monitor by keeping records and providing feedback and reinforcement to the individual on the changes and progress [12,47]. Using specific, proximal goals that are achievable fosters mastery performance—the most powerful source of enhancing self-efficacy. The use of credible models to demonstrate behaviors (e.g., how to modify a recipe or cook low-fat) is another important source for self-efficacy enhancement. A setting in which this is easily implemented is group sessions for cooking demonstrations. Research has provided evidence across multiple behavior domains that increased self-efficacy is associated with improved outcomes—e.g., making healthy food choices [48], developing healthier eating habits such as reducing fat and sodium intake [49], increasing fruit and vegetable consumption [50], improving health-related behavior outcomes such as glucose control in type 2 diabetes [51], and sustaining weight loss maintenance [52,53].

The key SCT construct of reciprocal determinism means that a person can be both an agent for change and a responder to change [41]. Thus, changes in the environment, the examples of role models, and reinforcements can be used to promote healthier behavior. For example, Gase and colleagues reported that self-efficacy was a mediator of the association between perceived food environment and healthy eating in low-income population [54]. Others have reported that self-efficacy is a mediator of the relationship between adherence to dietary self-monitoring and weight loss outcomes [53]. This core construct is also central to the social ecological model, which is discussed later.

2 Self-Regulation

Self-regulation, part of SCT, posits that individuals seek control over important events in their lives by self-regulating their thoughts, behaviors, and environment to achieve their defined goals [40]. According to this theory, reciprocal interactions are assumed to occur among behaviors, environment, and personal factors [41]. Kanfer [55] noted that the major self-regulative mechanism operates through three main functions: self-monitoring, self-evaluation, and self-reinforcement. The key to this process is the behavioral strategy of self-monitoring. Individuals cannot influence their motivation and actions well unless they pay deliberate attention to their own performance, as well as the conditions under which they occur and their immediate and long-term effects [43]. Thus, success in self-regulation depends in part on the fidelity, consistency, and temporal proximity of self-monitoring [43].

Studies based on self-regulation showed positive effects on dietary behavior change, e.g., reductions in energy and saturated fat intake [56], increases in fruit consumption [57], and better fruit and vegetable intake [58]. Several large worksite nutrition programs have applied the constructs of SCT [59,60]. A multisite study, the Working Well Trial, used an intervention rooted in SCT, consumer information processing, and the stages of change [61].

In SCT, self-regulation-based interventions focused on improving eating habits and achieving and/or maintaining a healthy weight and lifestyle have emphasized the role of self-monitoring as a key behavior (e.g., food intake, physical activity, and weight) [62]. A systematic review of the self-monitoring literature found that there was a consistent significant association between dietary self-monitoring and weight loss [46]. A self-regulation intervention focused on daily self-weighing and monitoring of weight with set goals for weight loss maintenance demonstrated efficacy in a clinical trial [63–65].

The use of new technologies for self-monitoring diet, although more expensive than traditional pen-and-paper methods, may reduce the burden of recording [66]. Studies using personal digital assistants for self-monitoring demonstrated greater reduced energy, saturated fat intake, and weight change [56], increased vegetable and whole-grain intake in middle-aged and older adults [67], and reduced sodium intake in hemodialysis patients [68]. A behavioral weight loss trial that provided daily feedback to those using an electronic diary to self-monitor their diet showed

improved self-monitoring adherence and a higher proportion losing 5% of their baseline weight compared to those who did not receive the daily feedback message [56]. Recently developed diet-related application (apps) for use in self-monitoring on mobile devices integrate the constructs of self-regulation theory, which are helpful to promote healthy dietary behaviors, particularly regarding emphasis on goal-setting for limiting calories or for food group or nutrient intake [20,69]. Additionally, researchers are developing apps for mobile devices for self-monitoring of diet to promote dietary behavior changes. For example, a wearable computer, eButton, is able to capture digital pictures of food and estimate portion size [70,71]. A mobile phone-captured food image to identify food and volume can be analyzed to estimate portion size, energy, and nutrients consumed in real time [72]. Compared to traditional methods, children and adolescents have a strong preference for capturing food images with the use of technology, which has demonstrated improved cooperation and increased accuracy in reporting diet [73].

B Stages of Change

Long-term dietary change for disease prevention or risk reduction involves multiple actions and adaptations over time. Some people may not be ready to attempt changes, whereas others may have already begun to change their diet and eating habits. The construct of "stage of change" is a key element of the TTM of behavior change, and it proposes that people are at different stages of readiness to adopt healthful behaviors [74–76]. The construct of readiness to change, or stage of change, has been examined in dietary behavior research and found useful in explaining and predicting the adoption of dietary change [76–78], and has also been applied in the treatment of childhood obesity [78,79].

Stages of change is a heuristic model that describes a sequence of steps in successful behavior change: precontemplation (no recognition of need for or interest in change), contemplation (thinking about changing), preparation (planning for change), action (adopting new habits), and maintenance (ongoing practice of new, healthier behavior) [77]. People do not always move through the stages of change in a linear manner. They often recycle and repeat certain stages: e.g., individuals may relapse and go back to an earlier stage depending on their level of motivation and self-efficacy.

The stages of change model can be used both to help understand why individuals might not be ready to undertake dietary change and to improve the success of dietary interventions [31]. During the past two decades, there has been a substantial increase in research applying the stages of change model to dietary behavior [80–83]. Prospective intervention research examining employees' readiness to change their eating patterns has revealed "forward movement" across the stage continuum in worksite nutrition studies and has shown that changes in stage of change for healthy eating are significantly associated with dietary improvements [59].

Multiple validated tools are available to assess readiness to change [79,84]. Prior to development of these tools, patients were classified according to their stage of change by asking a few simple questions: are they interested in trying to change their eating habits, thinking about changing their diet, ready to begin a new eating plan, already making dietary changes, or trying to sustain changes they have been following for some time? By knowing their current stage, clinicians can help to determine the best approach to intervening—e.g., how much time to spend with the patient, whether to provide informational material or dramatic relief for the person to consider during the contemplative stage, whether to wait until the person is more ready to attempt making changes, or whether referral for in-depth dietary counseling when preparation and action is warranted.

Assessing the patient's current stage of change can lead to appropriate follow-up questions about past efforts to change, obstacles and challenges, and available resources for overcoming barriers. Evidence has suggested that interventions at different stages have different results and implications for care, and these may be gender specific [85]. People's initial stage of change may influence their participation in dietary interventions. Individuals who are initially in the later stages of change (preparation, action, and maintenance) tend to spend more time on complex behaviors such as dietary change [86,87]; they also report making more healthful food choices. A study that assessed readiness for change in adapting a plant-based diet found the majority of the participants identified themselves to be in the precontemplation stage, recognizing fewer benefits and more barriers to adopting a plant-based diet. Evidence demonstrated that the states of action and maintenance were associated with more positive changes in individuals with type 2 diabetes and parents of children with obesity [88,89]. Other reports using this theory revealed that individuals in the intervention group who were in the precontemplation or maintenance stage were more likely to achieve improved adherence to goal-setting compared to those in similar stages in the control group [81,90]. However, not all evidence supported the use of the TTM. Systematic reviews of stage-based interventions have questioned the effectiveness of TTM, providing important critiques and noting the limitations of the theory [91,92]. Some argue that the theory draws arbitrary lines differentiating stages, categorizing individuals to stable coherent plans in periods of instability and mixtures of constructs, while focusing change on conscious decision making and planning, which diverts attention away from human motivation [93].

Evidence supports limited long-term effects for dietary change under stages of change interventions because these behaviors are more complex and require a number of specific actions to influence self-efficacy and lifestyle change [91,94,95]. In addition, two systematic reviews of behavioral techniques for weight loss failed to identify stages of change strategies as a key intervention in either impaired mobility subjects [96] or in the elderly [97]. Thus, while Prochaska's theory is well known, and there is some evidence of effectiveness of its use with behavior change as related to weight management, it is neither extensive nor overwhelming. This may be due in part to the complexity of dietary behavior change and attributing change to a singular feature.

C Consumer Information Processing

People require information about how to choose nutritious foods in order to follow guidelines for healthy eating. A central premise of consumer information processing theory is that individuals can process only a limited amount of information at one-time [98]. People tend to seek only enough information to make a satisfactory choice. They develop heuristics, or rules of thumb, to help them make choices quickly within their limited information processing capacity. The nutrition information environment is often complex and confusing; especially when programs rely heavily on print nutrition educational materials that may be written at a level higher than the audience can comprehend.

Several elements of consumer information processing theory can be applied in dietary interventions. Seemingly unrelated aspects have influence: in a survey, consumers reported associating yellow, blue, green, and red colored labels to be healthy foods and heather, pink, and celadon suggesting something artificial and seemingly unhealthy [99]. Front of package messages may be attended to by consumers more than Nutrition Facts Panels when signage drew attention to them [100]. Messages that are food focused rather than nutrient focused may be particularly helpful [101,102]. Nutrition information is most helpful when it is tailored to the comprehension level of the audience, matched to their lifestyles and experience, and is either portable or available at or near the point of food selection [102–104]. Nutrition labeling had an impact with evidence of interpretive labeling like "traffic light" design endorsed by multiple researchers as most effective [105–110]. Other data suggested other simplistic interventions were useful: simple happy versus sad emoticons on products reduced ambiguity for consumers [111].

An analysis of the National Health and Nutrition Examination Survey 2005 data revealed that four out of five Americans, age 16 years or older, were aware of one of the three federal dietary guidance efforts—the Food Guide Pyramid, 5 A Day Program, and Dietary Guidelines for Americans (DGAs); however, there were significant differences when comparing race and ethnic groups (non-Hispanic white Americans were more familiar), as well as among the different levels of education and income groups. MyPlate introduced by USDA in 2011 has had modest acceptance. Nestle USA partnered with Harris Interactive to conduct a survey in 2013 to gage consumer knowledge of MyPlate and the Dietary Guidelines. Their data showed 41% of consumers were aware of MyPlate or ChooseMyPlate.gov, 92% of consumers had not visited ChooseMyPlate.gov, and 87% of consumers indicated interest in learning about simple ways to follow the DGA [112]. Thus while 59% were unaware of MyPlate, most indicated interest in eating more healthfully. Cluss et al. [113] assessed understanding of the Food Guide Pyramid of parents with children in an obesity treatment program from a large urban pediatric care setting and found "black race and lower education within a low-income sample were independently related to misidentification of low nutrition and high caloric content foods" [113]. Better results of consumer awareness of MyPlate were found in a smaller sample, with 40% reporting that it had influenced their choices [114]. In a review of interventions targeting at young adults in educational institutions, information such as nutrition messages and labeling resulted in positive behavior change [115].

These reports in summation indicate nutrition education via MyPlate can be effective but there is a continued need to educate all individuals on eating a better quality diet, including those in minority groups, those with lower socioeconomic status and low literacy levels about nutrition and how to read dietary information.

The Patient Protection and Affordable Care Act of 2010 mandated menu labeling to disclose nutrient information for standard menu items for chain restaurants or similar retail food establishments that are part of a chain with 20 or more locations [116]. The objective of this initiative was to improve access to consumer information so that individuals can make informed decisions and select more healthful menu options [117,118]. The Federal Register notes some studies have shown that individuals consume fewer calories when menus have information about calories on the menus, critical for Americans who now consume 30% of meals outside the home. Two meta-analyses of the effects of nutrition labeling on menus reported similar results: labeling had a modest effect on calorie consumption but remained a low-cost strategy which needed further study to understand what is most effective [119,120].

Knowledge about which foods to choose, and how much to consume, on a therapeutic diet, are the *sine qua non* of dietary adherence. However, knowledge of how to use nutrition information and the skills to choose or prepare healthful foods are insufficient for behavior change without motivation and support. Furthermore, patients

with low literacy skills and language barriers may require more explanations and fewer printed materials, thus posing important challenges [121]; those with better education or motivation for health concerns may increase likelihood of using nutrition labels. Community studies involving participants with low literacy levels and multicultural backgrounds demonstrated successful dietary changes by administering the intervention through mixed multimedia and audiovisuals [122]. Those successfully conveying education through print materials suggest using simple messages, one- or two-syllable words, large print, and, when necessary, the participant's first language [121–124]. It appears as interpretive labels, most commonly the traffic light system, whose colors are universally recognized, are consistent in showing better effectiveness in changing consumer behavior.

D Theory of Planned Behavior

Often people's food choices are influenced by how they view the actions they are considering and whether they believe important others such as family members or peers would approve or disapprove of their behavior. The TPB, which evolved from its predecessor, the theory of reasoned action, focuses on the relationships between behavior and beliefs, attitudes, subjective norms, and intentions [80]. The concept of perceived behavioral control involves the belief about whether one can control his or her performance of a behavior [125]; i.e., people may feel motivated if they believe they "can do it." A central assumption of TPB is that "behavioral intentions" are the most important determinants of behavior [126].

TPB has been applied widely to help understand and explain many types of behaviors, including eating behavior [127]. The core constructs of TPB have been found to explain fast-food consumption behaviors among middle-school students whose behaviors were mostly influenced by subjective norms [128] and saturated fat intake among adults affected by habit strength and behavior intention [129]. The TPB has also been found to predict intention to dietary change [130] and healthy eating such as fruit and vegetable intake [46], eating breakfast, cooking homemade meals, and eating together [131] and to be more effective in predicting expectation than intention or desire for controlling weight [132]. Behavioral intentions are important and central to TPB; however, there has been some concern that they are still too far removed to be good predictors of actual behavior. The concept of *implementation intentions* involves encouraging individuals receiving an intervention to be specific about how they would change. One study found that providing implementation intention prompts led to greater weight reduction in a commercial weight loss program [133]. Overall, while the TPB has been the focus on limited study for more than 30 years, recent reviews note that "the potential of the TPB as a model either to understand 'food choice' behaviors or serve as a basis for intervention development, appears limited" [130]. The authors emphasize the complexity of determinants of eating behaviors and the importance of considering alternate behavioral models when planning interventions.

E Multiattribute Utility Theory

Both health professionals and marketers recognize that people seek the things they like and that give them pleasure, and that they take action to obtain these things. Identifying those concerns that are most important to a person's decision about performing a specific behavior can lead to the development of effective interventions and decision aides to promote desirable behaviors [12]. MAU is a form of value expectancy theory that aims to specify how people define and evaluate the elements of decision-making about performing a specific behavior. Key elements of value expectancy theory are the valence, or importance, of a particular feature of a behavior or product and the expectancy, or subjective probability that a given consequence will occur if the behavior is performed [134].

MAU is a form of value expectancy theory with particular relevance to understand influences on food choice and changes in eating habits. MAU posits that people evaluate decisions based on multiple attributes and somehow consciously or unconsciously weigh the alternatives before deciding what actions to take. A widely cited report used MAU as the framework for analyzing surveys of a national sample of 2967 adults. The study identified several key factors that appear to be important in food selection: taste, nutrition, cost, convenience, and weight control [23]. For the general public, taste has been reported to be the most important influence on food choice, followed by cost [23]. Understanding the relative importance of various concerns to individuals can guide the design of nutrition counseling and nutrition education programs. For example, by designing and promoting a nutritious diet as tasty, an intervention might be more successful than if it is presented primarily as nutritious or inexpensive. No published reports have explicitly applied MAU to design a dietary intervention, but a project that used tailored messages based on alternative food choices and the attributes among them was found to be effective for increasing dietary fiber consumption; it was based on the behavioral alternatives model, which has many similar features to MAU [135].

F Social Ecological Model

The social ecological model helps researchers understand factors affecting behavior and also provides guidance for

developing successful programs through social environments. The social ecological model emphasizes multiple levels of influence (e.g., individual or intrapersonal, interpersonal, organizational, community, and public policy) and the idea that behaviors both shape and are shaped by the social environment [24,25].

The principles of the social ecological model are consistent with SCT concepts that suggest creating an environment conducive to change is important to making it easier to adopt healthy behaviors [40]. Given the widespread problems of over nutrition in developed countries, more attention is being focused on increasing the health-promoting features of communities and neighborhoods, reducing the ubiquity of high-calorie, high-fat food choices [30,136] as well as promoting high fruit and vegetable intake [137].

The social ecological model is useful to guide intervention research related to changing dietary habits because of its focus on multilevel linkages [17]. This model is often discussed in the context of the obesity epidemic [30]. Also, it has been endorsed as a foundation for diet-related behavior interventions in children considering the contextual influences on childhood obesity, and its focus on prevention is embedded in the home, school/community, and society at large [138–140]. Health promotion programs that include a focus on home [141], worksite [142,143], and church [144] environments can improve eating habits and weight management of adults. Studies have found that socio-ecological resources mediated the lifestyle intervention effects on saturated fat consumption among postmenopausal women with type 2 diabetes [145,146]. The literature also suggests that this model can provide guidance for intervention development for pregnant women [147], low-income African Americans to support healthy eating habits [148], and childhood obesity [149].

Overall, in the past two decades, there has been an increase in published research applying theoretical models to longitudinal studies and clinical trials testing interventions for diet-related behavior change, particularly applying SCT, stages of change, and the social ecological model [130,150,151]. Numerous studies have examined the determinants of eating behavior using coherent theoretical frameworks and constructs. There continues to be a need for more studies using longitudinal designs studying families, the changing food roles in families as well as the changing food environment.

G Selecting an Appropriate Theoretical Model or Models

Effective nutrition intervention depends on marshaling the most appropriate theory and practice strategies for a given situation [152]. Different theories are best suited to different individuals and situations. For example, when attempting to overcome a patient's personal barriers to changing his or her diet to reduce his or her cholesterol level, TPB may be useful. The stages of change model may be especially useful in developing diabetes education interventions. When trying to teach low literacy patients how to choose and prepare healthy foods, consumer information processing may be more suitable. The choice of the most fitting theory or theories should begin with identifying the problem, goal, and units of practice [12,152], not with simply selecting a theoretical framework because it is intriguing or familiar.

With regard to practical application, theories are often judged in the context of activities of fellow practitioners. To apply the criterion of usefulness to a theory, most providers are concerned with whether it is consistent with everyday observations [12,152]. In contrast, researchers usually make scientific judgments of how well a theory conforms to observable reality when empirically tested. Patient educators should review the research literature periodically to supplement their firsthand experience and that of their colleagues.

A central premise in understanding the influences on health behavior and applying them to patient education is that one can gain an understanding of a patient through an interview or written assessment and better focus on the individual's readiness, self-efficacy, knowledge level, and so on. Clearly, it is necessary to select a "short list" of factors to evaluate, and this may differ depending on clinical risk factors or a patient's history. Once there is a good understanding of that person's cognitive and/or behavioral situation, the intervention can be personalized or tailored. Tailored messages and feedback or delivering these messages and feedback through mHealth [47,153,154] have been found to be promising strategies for encouraging healthful behavior changes in primary care, community, and home-based settings [155]. The challenge of successfully applying theoretical frameworks in nutrition programs involves evaluating the frameworks and their key concepts in terms of both conceptual relevance and practical value [14]. In recent years, the focus on home self-management of chronic disease such as diabetes and cardiovascular disease has increased. Nutrition professionals can assist in the development of these comprehensive approaches to patient care. The integration of multiple theories into a comprehensive model tailored for a given individual by frequent assessments can guide the implementation of best-practice interventions, and improve patient outcomes [156]. Psychosocial issues to consider during program development and implementation include social/cultural factors, socioeconomic status, access to community/health care resources, literacy level, personal experience, available support systems, and the

individual's knowledge and skills to manage the condition for which treatment is designed [157].

VII CONSTRUCTS AND ISSUES ACROSS THEORIES

It is important to bear in mind that the various theories that can be used for dietary interventions are not mutually exclusive. Not surprisingly, they share several constructs and common issues. It is often challenging to sort out the key issues in various models. This section focuses on important issues and constructs across models, the first of which is that successful dietary behavior change depends on a sound understanding of the patient's, or consumer's, view of the world.

A The Patient's View of the World: Perceptions, Cognitions, Emotions, and Habits

For health professionals who work with patients and provide them with advice on health and lifestyle, adherence to treatment is often disappointingly poor, even in response to relatively simple medical advice. Such poor adherence often arises because patients do not have the necessary behavioral skills to make changes to their diet. Following a heart attack, e.g., patients might well understand the importance of changing their lifestyle but are unable to make the suggested changes. There will be other circumstances in which patients might not understand the importance of suggested changes and may even believe that the recommended changes pose an additional risk to their health. Indeed, motivational interviewing seeks to elicit the ambivalence patients have about making changes, the source of which may be inaccurate or emotionally based perceptions. In still other circumstances, patients might be experiencing depression or anxiety, such that emotional dysfunction will be a major barrier to adherence. In addition, a longitudinal study demonstrated that adherence to the dietary intervention protocol declined steadily even during the intervention period as the frequency of contact declined, which suggests that it is difficult for participants to sustain the behavior changes without ongoing reinforcement [158].

Traditionally, it has been assumed that the relationship between knowledge, attitudes, and behavior is a simple and direct one. Indeed, throughout the years, many prevention and patient education programs have been based on the premise that if people understand the health consequences of a particular behavior, they will modify it accordingly. Moreover, the argument goes, if people have a negative attitude toward an existing lifestyle practice and a positive attitude toward change, they will make healthful changes. However, it is now known from research conducted during approximately the past 30 years that the relationships among knowledge, awareness of the need to change, intention to change, and an actual change in behavior are very complex.

Ideally, each patient should be treated as an individual with unique circumstances and health history. Still, epidemiological research and clinical trials indicate that certain demographic subgroups differ in terms of risk factors, eating behaviors, and dietary patterns [159,160]. Understanding these population trends can help prepare a provider to work with various types of patients. For example, younger persons may feel invulnerable to coronary events, and older adults may be managing multiple chronic conditions. An active middle-aged professional may place returning to his previous level of activity above important health protective actions. These examples serve as a reminder to be sensitive to group patterns but to avoid stereotyping in the absence of firsthand evidence about an individual. Within this general context, various theories and models can guide the search for effective ways to reach and positively motivate individuals.

B Behavior Change as a Process

Sustained health behavior change involves multiple actions and adaptations over time. Some people may not be ready to attempt changes, some may be thinking about attempting change, and others may have already begun implementing behavioral modifications. One central issue that has gained wide acceptance in recent years is the simple notion that behavior change is a process, not an event. Rather, it is important to think of the change process as one that occurs in stages. It is not a question of someone deciding one day to change his or her diet and the next day becoming a low-fat eater for life. Likewise, most people will not be able to dramatically change their eating habits all at once. The idea that behavior change occurs in a number of steps is not particularly new, but it has gained wider recognition in recent years. Indeed, various multistage theories of behavior change date back more than 50 years to the work of Lewin, McGuire, Weinstein, Marlatt and Gordon, and others [12,161,162].

The notion of readiness to change, or stage of change, has been examined in health behavior research and found to be useful in explaining and predicting a variety of behaviors. Prochaska, Velicer, DiClemente, and colleagues have been leaders in beginning to formally identify the dynamics and structure of change that underlie both self-mediated and clinically facilitated health behavior change. The construct of stage of change (described previously) is a key element of their TTM of behavior change, and it proposes that people are at different stages of readiness to adopt healthful behaviors [163,164].

Although the stages of change construct cuts across various circumstances of individuals who need to change or want to change, other theories also address these processes. Here, we discuss various models to illustrate four key concerns in understanding the process of behavior change: (1) motivation versus intention, (2) intention versus action, (3) changing behavior versus maintaining behavior change, and (4) the role of bio-behavioral factors.

1 Motivation Versus Intention

Behavior change is challenging for most people even if they are highly motivated to change. As previously noted, the set of relationships between knowledge, awareness of the need to change, intention to change, and an actual change in behavior are very complex. For individuals who are coping with disease and illness, and who are often having to make very significant changes to their lifestyle and other aspects of their lives, this challenge is even greater. According to the TTM, people in precontemplation are neither motivated nor planning to change, those in contemplation intend to change, and those in preparation are acting on their intentions by taking specific steps toward the action of change [164].

2 Intention Versus Action

The TTM makes a clear distinction between the stages of contemplation and preparation and overt action [163,164]. A further application of this distinction comes from the TPB [128], which proposes that intentions are the best predictor of behavior [125,165]. However, researchers are increasingly focusing attention on "implementation intentions" as being more proximal and even better predictors of behavior and behavior change [133].

3 Changing Behaviors Versus Maintaining Behavior Change

Even when there is good initial adherence to a lifestyle change program, such as changing diet or eating habits, maintenance of long-term behavior changes is challenging [158,159]. Thus, it has become clear to researchers and clinicians that undertaking initial behavior change and maintaining behavior change require different types of strategies. The TTM distinction between "action" and "maintenance" stages implicitly addresses this phenomenon [164]. Another model that is not described in detail here, Marlatt and Gordon's relapse prevention model, specifically focuses on strategies for dealing with maintenance of a recently changed behavior [161]. It involves developing self-management, coping strategies and establishing new behavior patterns that emphasize perceived control and environmental management as well as improve self-efficacy. These strategies are an eclectic mix drawn from SCT [40], the TPB [125], and applied behavioral analysis—the forerunners of the stages of change model.

C Bio-behavioral Factors

The behavioral and social theories described thus far have some important limitations, many of which are only now beginning to be understood. Notably, for some health behaviors, especially addictive or addiction-like behaviors, there are other important determinants of behavior, which may be physiological and/or metabolic. Among the best known are the addictive effects of nicotine, alcohol, and some drugs. Physiologic factors increase psychological cravings and create withdrawal syndromes that may impede even highly motivated persons from changing their behaviors (e.g., quitting smoking and not consuming alcoholic beverages). Some behavior changes, such as weight loss, also affect energy metabolism and make long-term risk factor reduction an even greater challenge than it would be if it depended on cognitive−behavioral factors alone. Research on the psychobiology of appetite offers intriguing possibilities for understanding bio-behavioral models of food intake, including food addiction.

D Barriers to Actions, Pros and Cons, and Decisional Balance

According to SCT [40], a central determinant of behavior involves the interaction between individuals and their environments. Behavior and environment are said to continuously interact and influence one another, which is known as the principle of reciprocal determinism. The concept of barriers to action, or perceived barriers, can be found in several theories of health behavior, either explicitly or as an application. It is part of SCT [40] and the TPB [125]. In the TTM, there are parallel constructs labeled as the "pros" (the benefits of change) and "cons" (the costs of change) [164]. Taken together, these constructs are known as "decisional balance."

The idea that individuals engage in relative weighing of the pros and cons has its origins in Janis and Mann's model of decision-making, published in their seminal book more than 40 years ago [166], although the idea had emerged much earlier in social psychological discourse. Lewin's idea of force field analysis [167] and other work on persuasion and decision counseling by Janis and Mann predated that important work. Indeed, this notion is basic to models of rational decision-making, in which people intellectually consider the advantages and disadvantages, obstacles and facilitators, barriers and benefits, or pros and cons of engaging in a particular action.

E Control over Behavior and Health: Control Beliefs and Self-Efficacy

Sometimes, "control beliefs" and self-efficacy prevent people from achieving better health. These deterrents to positive health behavior change are common, and they can be found in several models of health behavior, including SCT, TPB, and relapse prevention. One of the most important challenges for these models—and ultimately for health professionals who apply them—is to enhance perceived behavioral control and increase self-efficacy, thereby improving patients' motivation and persistence in the face of obstacles.

VIII IMPLICATIONS AND OPPORTUNITIES

Theory and research suggest that the most effective dietary interventions are those that use multiple strategies and aim to achieve multiple goals of awareness, information transmission, skill development, and supportive environments and policies [81]. The range of dietary intervention tools and techniques is extensive and varied. Programs will differ based on their goals and objectives, the needs of clients, and the available resources, staff, and expertise. Dietary interventions can stand alone or be part of broader, multicomponent, and multiple-focus health promotion and patient education programs.

What can be expected? Program design relates closely to what one can expect in terms of results. In general, minimally intensive intervention efforts such as one-time group education sessions can reach large audiences but seldom lead to behavior changes. More intensive programs typically appeal to at-risk or motivated groups; cost more to offer; and can achieve relatively greater changes in knowledge, attitudes, and eating habits [168].

Dietary interventions must be sensitive to audience and contextual factors. Food selection decisions are made for many reasons other than just nutrition: taste, cost, convenience, and cultural factors all play significant roles [23]. Dietary change strategies must take these issues into consideration. The health promotion motto "know your audience" has a true and valuable meaning. Planning processes can consider multiple theories in a systematic way through approaches such as intervention mapping [169]. Intervention programs should be developed on the basis of the needs, behaviors, motivations, and desires of target audience [170,171].

Furthermore, change is incremental. Many people have practiced a lifetime of less than optimal diet-related behaviors. It is unreasonable to expect that significant and lasting changes will occur during the course of a program that lasts only a few months. Programs need to nudge participants along the continuum of change, being sure to be just in front of those most ready to change with attractive, innovative offerings.

In population-focused programs, it appears to be of limited value to adopt a program solely oriented toward modifying individual choice (e.g., teaching and persuading individuals to choose low-fat dairy products). A more productive strategy that supports and facilitates healthy behavior changes would also include environmental change efforts, such as expanding the availability of more nutritious food choices [2,24,25,136]. When this is done, along with individual skill training, long-lasting and meaningful changes can be achieved.

Finally, when planning interventions, we need to strive to be creative. Dietary interventions should be as entertaining and engaging as the other activities with which they are competing. People will want to participate if they can have fun with the nutrition programs. Communication technologies have opened up many new channels for engaging people's interest in better nutrition. The communication of nutrition information, no matter how important it is to good health, is secondary to attracting and retaining the interest and enthusiasm of the audience. The mHealth interventions which incorporate constructs of different *theoretical* models need to be further studied and evaluated for treatment efficacy [154,172].

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Chapter 10

Nutrition Intervention: Lessons From Clinical Trials

Linda G. Snetselaar[1] and Linda M. Delahanty[2]
[1]University of Iowa, Iowa City, IA, United States, [2]Harvard Medical School, Boston, MA, United States

I INTRODUCTION

The modification of dietary patterns to prevent and optimize the management of chronic disease has been traditionally perceived as a difficult and challenging task. However, much has been learned since the 1980s about how to successfully modify eating patterns. For example, in clinical trials, several diet intervention studies that focused on the prevention of cancer or cardiovascular disease have demonstrated the feasibility of reducing dietary fat intake in targeted groups. In addition, complex dietary modifications testing the effect of diet on the progression of renal disease have also been successfully achieved.

II COMMON COMPONENTS OF DIETARY INTERVENTIONS IN CLINICAL TRIALS

A Study Design

A frequently used research design is the randomized control trial (RCT) in which the study begins by screening those study participants who meet the eligibility criteria. Often, very large numbers of participants are screened to arrive at the final number of participants who meet these criteria. For those who are screened into the study, each participant is randomly assigned to either dietary intervention or control, sometimes labeled the usual care group.

B Recruitment

Recruitment for an RCT can involve a variety of strategies, including newspaper advertisements, posters at pharmacies and in doctors' offices, booths at county and state fairs, mass mailings, and presentations to appropriate groups who may become study participants. It is wise to have a number of recruitment strategies. If one fails, others can be initiated to achieve the targeted sample size goal. Although recruiting the number of study participants needed for optimum sample size is an important element of an RCT, recruiting participants into a study who will remain with the study for the entire dietary intervention is equally important. Carefully reviewing the required internal review board-approved study participant consent is crucial. If a potential study participant has the following responses when the study is described, he or she may not be an appropriate candidate for the study:

- Hesitant about being willing to be a part of either the control (usual care) or the intervention group and voices that only the intervention group is best for him or her;
- Concerned about the time that the study intervention group will require;
- Unsure about getting supervisor-approved time off to participate in the mandatory intervention group activities;
- Adamant to be a part of the study when the study has another objective that is counter to this rationale for study participation;
- Concerned about being able to continue the study for the entire time it is scheduled to run;
- Unsure about spouse or significant other support for study activities;
- Unwilling to comply with required activities explicitly stated in the consent form;
- Concerned about travel time needed to participate in the study.

Placing a study participant in a study when these criteria exist may mean that the number of dropouts

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in the study increases. Losing a study participant following randomization reduces the sample size and compromises study power to show a difference between the intervention and the control group. When randomized study participants drop out of a study, this is equivalent to under-recruiting and not meeting required study sample numbers. Study participants who drop out of a study cannot be replaced.

C Intervention Design

Study interventions begin with an initial hypothesis. The following are two examples:

- This study will compare the dietary intervention and control groups using data on the number of study participants who have a change in waist circumference of 4 or 5 inches over a 2-year intervention period.
- Following a 3-year dietary intervention study, participants will have a change in hemoglobin A1c that is double that of the control group.

These hypotheses are designed to provide a comparison of the intervention and control groups. Note that each hypothesis has a numeric difference. Without this difference, the study will not show that the dietary intervention did make a difference.

The strategy for the dietary intervention should have a basis in other studies that may have had smaller numbers and short periods of intervention time compared to those of the RCT. In addition, the RCT should be backed by a pilot study to show the ability to achieve the numeric changes that the original hypothesis indicates.

A more recent study design that can benefit from the older RCT studies is the community-based participatory research study. This more community-focused study requires that community participants who will be involved in implementing the study have a voice in its design. For this study, participants will need to be involved in the beginning phases of the study design and the development of implementation strategies. Without their involvement, the study will not be truly participatory. Whereas RCTs are more structured and have many checks and balances, this more formalized method of data collection can be more difficult in a community setting. For example, absentee data collection in a school setting may be very unstructured, with different personnel collecting the data and little standardization in the recording of the reasons for a student being absent. To mimic a more structured RCT, rules and guidelines that will allow for consistent categorization of absenteeism are required. This means working with school personnel—those collecting the data and those eventually entering data—to ensure that everyone is of equal understanding with a new policy or procedure for data collection.

III CONCEPTUAL MODELS OF MOTIVATION

Interventions are developed around models to provide conceptual designs for motivation for positive directions in dietary adherence. Theories for behavior change are extensive, and some offer techniques that are practical for use in nutrition counseling. This section discusses theories that describe models of motivation.

A Self-Regulation Theory

This theory, originally described by Kanfer, states that behavior is regulated by cycles that involve self-monitoring, comparing goal achievement with expectations, and correcting the course of action when the goal is not met [1,2]. To change dietary behavior, a person seeks to increase knowledge of the discrepancy between current status and the identified goal. Two ways to accomplish this are: (1) to increase the awareness of current status (e.g., through feedback such as dietary self-monitoring) or (2) to change the goal to make it more attainable. In conflict situations, when a goal is desired and yet not seen as important enough to strive to attain, ambivalence (feeling at least two different ways about something) is a normal, key obstacle to dietary change.

An example might be used of the double-sided reflection as described in this example.

Client: I know that I should be doing a better job of exercising and reducing my carbohydrate intake, but I love the foods that are high in carbohydrate and just cannot give them up.

Dietitian: You know that exercising and reducing carbohydrate loaded foods would be good for you, but you have great difficulty giving them up.

B Rokeach's Value Theory

Studies in persons who have undergone sudden transformation shifts in behavior show that personality is organized around concentric layers [3]. An individual's attitudes, numbering in the thousands, represent an organizational series of steps inward. More central are our beliefs, and even more central are our core personal values. The most central is the sense of personal identity. The more central the shift, the more likely the resulting behavior change will be maintained over time. In order to move forward with change often discovering what a person values can be a step in a positive direction. An example follows:

Client: I love playing with my grandchildren and do not want to have complications due to poor diabetes control making that impossible.

Counselor: Playing with your grandchildren is something that you value greatly.

C Health Belief Model

The health belief model attempts to explain and predict health behaviors by focusing on the attitudes and beliefs of individuals. The key variables of the health belief model are as follows [4]:

Degree of perceived risk of a disease. This variable includes perceived susceptibility of contracting a health condition associated with lack of a healthy diet and its perceived severity once the disease is contracted.

Perceived benefits of diet adherence. A second benefit is the believed effectiveness of dietary strategies designed to help reduce the threat of disease.

Perceived barriers to diet adherence. This variable includes potential negative consequences that may result from changing dietary patterns, including physical (weight gain or loss), psychological (lack of spontaneity in food selection), and financial demands (cost of new foods).

Cues to action. Events that motivate people to take action in changing their dietary habits are crucial determinants of change.

Self-efficacy. A very important variable is the belief in being able to successfully execute the dietary behavior required to produce the desired outcomes [5–7].

Other variables. Demographic, sociopsychological, and structural variables affect an individual's perceptions of dietary change and thus indirectly influence his or her ability to sustain new eating behaviors.

Motivation for change depends on the presence of a sufficient degree of perceived risk in combination with sufficient self-efficacy relative to achieving dietary change. Perceived risk without self-efficacy tends to result in defensive cognitive coping, such as denial and rationalization, rather than behavior change.

D Decisional Balance

The classic Janis and Mann decisional balance model [8] was a rational view, describing a decision as a process of weighing cognitively the pros and cons of change. Change depends on the pros of change outweighing the cons. The counselor who is adept at reflective listening skills, designed to assist the client in reviewing those ideas that result in an optimum final choice, can facilitate determining the strengths and weaknesses of a decision.

E Transtheoretical Model

This model indicates that individuals change depending on where they are in conceptual readiness to modify their behavior. Prochaska and DiClemente were the forerunners of this theory that focuses on the concept that behavioral change happens in stages of motivation as clients move to a more healthful lifestyle.

The model postulates that both cessation of high-risk behaviors and the acquisition of healthy behaviors involve progression through five stages of change: precontemplation, contemplation, preparation, action, and maintenance [9]. The precontemplation stage is characterized by having no intention of changing the behavior in question in the foreseeable future. People in this stage tend to be unaware that they have a problem and are resistant to efforts to modify the behavior in question. Contemplation is characterized by awareness that the particular behavior is a problem and requires serious consideration about resolving the problem but having no commitment to take action in the near future. The preparation stage is the stage of decision making. The person has made a commitment to take action within the next 30 days and is already making small behavioral changes. In the action stage, clients make notable overt efforts to change. Maintenance involves working to stabilize the particular behavior change and avoid relapse for the next 6 months. People do not simply progress through the stages in a straight line; they may recycle by relapsing and repeating stage progressions, and they can enter or exit at any point [10,11]. This transtheoretical model has been shown to generalize across a broad range of problem behaviors (including diet, exercise, and weight control) and populations [10].

Integral to the stages of change model is a "standard" or goal for the behavior that has been proven to produce results. For example, the goal of the national cholesterol education program (NCEP) therapeutic lifestyle changes (TLC) diet for a dietary saturated fat intake of less than 7% of energy and less than 200 mg cholesterol per day was established based on research showing associated reduction in serum low-density lipoprotein cholesterol concentration. Stages of change techniques help individuals evaluate current behaviors against a defined standard and then assess their readiness to change. The objective is to help patients achieve the standard for the behavior; however, incremental goals are often required. Fortunately, many nutrition interventions in clinical nutrition show benefits associated with stepwise progression to the goal.

If dietitians use the stages of change model as a basic technique for approaching nutrition counseling sessions in a research study, it often requires a modification in their

teaching and counseling style [12]. The focus of attention shifts from establishing an agenda to merely educate the individual and shifts toward an approach that assesses the status of the individual as a basis for tailoring the counseling session. For example, instructing a client with hypercholesterolemia who is clearly in the precontemplation stage on an NCEP TLC diet will be less effective than discussing the risks associated with high cholesterol levels and the benefits of reducing the saturated fat content of the diet. Although the counselor may feel an obligation to impart specific information, mismatching stages and interventions could break rapport and may lead the client to avoid further follow-up sessions.

F Self-Determination Theory

The self-determination theory is based on the concept that humans have an innate tendency for personal growth. The idea behind the self-determination theory is the question of how people internalize and integrate external factors that affect their motivation to change. Eventually, they will come to self-regulate their behaviors moving toward autonomy in actions in their daily lives. The use of reflections and affirmations can be helpful in achieving this autonomy in behavior change. Motivational interviewing (MI), although not considered a theory but rather a communications style, has been connected to this self-determination theory and uses reflections and affirmations as tools to achieve this autonomy.

This theory can be used when it seems that the client is not ready to make a change (precontemplation). Using MI can result in movement toward change talk and the eventual contemplation of behavior change followed by action in making a change.

MI is a client-centered communication style designed to build commitment and reach a decision to change [13]. It focuses on increasing the clients intrinsic motivation to change and self-confidence in their ability to do so. Results from several studies support the use of MI to improve glycemic control and enhance weight loss, particularly for those who are struggling with weight loss or have hit a weight loss plateau. The increased weight loss with MI appears to be mediated by enhanced treatment engagement and program adherence [14].

MI involves an interview process that elicits "change talk" or personally relevant reasons that support movement in the direction of healthful behavior change. "Change talk" may include expression of a concern, recognition of a problem, optimism about change, or an intention to change [14].

The goal of MI is to increase the frequency, range, and strength of client "change talk" and to minimize "sustained" talk where the focus is on maintaining current unhealthy behaviors. Although MI acknowledges that ambivalence is normal and exists among most people considering a behavior change, it does not seek to pull for or emphasize the reasons to stay the same (the disadvantages). This is because people who talk more about reasons not to change, do not change, whereas, those who express more "change talk" have a greater likelihood of making targeted behavior changes [15]. When clients hear themselves articulate why they want to change, it has a motivating effect.

Thus, rather than actively eliciting the advantages and disadvantages of behavior change, MI focuses on (1) asking strategic open-ended questions:

> What might a benefit of dietary change be to you?
> How do you think you would feel when you reach your goal?
> What do you see as the next step?

and (2) responding by spending a short time recognizing and acknowledging a client's reasons for staying the same but spending the most of the time emphasizing personalized reasons for change. Dietitians can use the OARS approach to increase the proportion of "change talk" that a client offers [14].

1 The OARS Approach

The OARS approach is described below:

> O: *Open questions*: Ask for more detail about the personal reasons for change (advantages of behavior change). Use the questions "how" and "what" or the statement "Tell me more. . .."
> A: *Affirmation*: Comment positively on the client's "change talk" statements.
> R: *Reflection*: Use reflective statements to further strengthen client's "change talk."
> S: *Strategic summary*: Collect a bouquet of all the "change talk," put it together, and give it back to the client. When all of the personal reasons are grouped together and reflected back to the client in a succinct summary, it can be powerfully motivating [14].
> *Dietitian*: "What makes you want to _____ (reduce fat intake, lose weight, lower blood sugars) right now?" (O)
> *Client*: "I'd really like to be on less medications and I know if I lose weight and increase my activity, then I might be able to reduce or get off of some of these medications." "I've had a hard time focusing because of other family stresses. I tend to eat when I am worried but I am trying to walk to help my stress."
> *Dietitian*: "You have done a wonderful job of taking all of your medications (A), but it concerns you that you are on so many different pills." (R)
> *Client*: "The more medications that I take, the less healthy I feel. All of these medications have side effects."

Dietitian: "So for you, a major benefit of losing weight and increasing your activity is to reduce the amount of medications that you take and to feel healthier as well." (R)
Client: "Yes that is right!"
Dietitian: "The fact that you've started walking to relieve stress instead of eating for stress is a very positive step that you have made toward your goal." (A)
Client: "Yes, I want to keep that up. I noticed that my energy level is better after I walk."
Dietitian: "You seem pleased that you have been able to fit walking into your schedule and that it is a constructive way to deal with your stress. It also seems to energize you." (R)
Client: "Yes I really want to try to do more."
Dietitian: "That sounds good. What are you ready to work on?" (O)
Client: "I would like to start using frozen proportioned meals for dinner 4 times per week. This will save me some time and help me eat less at dinner."
Dietitian: "That sounds like a really good idea because it will save you time, help you lose weight and get closer to your goal of being on less medication (A). Out of 100% confidence, how confident are you that you will be able to eat frozen dinners 4 days per week?" (O)
Client: "80% confident. I can do it."
Dietitian: "So, you definitely would like to be on less medication and you believe that losing weight and increasing your activity level is the key (A). It is clear that the walking that you have been doing energizes you and helps manage your stress. You feel that the next step is to use frozen dinners 4 times per week and that this is doable and will help you lose weight." (S)
Client: "I definitely feel I can do this. I can pick up the frozen dinners on the way home."

2 Elicit-Provide-Elicit to Provide Information and Advice

In addition to OARS, Miller and Rollnick identify the process of informing and advising. This process involves the concept of elicit-provide-elicit [16]. Often it can be used when a client has a mistaken idea about diet and disease.

Dietitian: What do you know or have heard about carbohydrates in food and their effect on diabetes control? (E)

Client: I try to eat very few foods with high carbs because I know that all carb is bad for you.

Dietitian: This is probably different from what you have heard but some carbs are fine for you to eat even though you have diabetes. (P)

Client: Oh, really.

Dietitian: What might this information do to alter what you do in selecting carbohydrate containing foods? (E)

Client: I will pay attention to the types of carbohydrate foods I select, but not try to avoid them completely.

3 FRAMES Strategy

Miller and Rollnick [17] suggested that the following specific motivational techniques can be combined to achieve an effective MI strategy (FRAMES):

Feedback: Clearly discuss the client's current health situation and risks and explain results of objective tests; share observations based on food records and weight trends. Clarify goals by comparing feedback on the client's current situation to some standard, and set goals toward that standard that are realistic and attainable.
Responsibility: Emphasize that it is the client's responsibility to change.
Advice: Clearly identify the problem or risk area, explain why change is important, and advocate specific change.
Menu: Offer the client a menu of alternative strategies for changing eating habits. Offering each client a range of options allows the individual to select strategies that match his or her particular situation and enhance the sense of perceived personal choice.
Empathy: Show warmth, respect, support, caring, concern, understanding, commitment, and active interest through attentive listening skills.
Self-efficacy: Reinforce the client's self-efficacy via competency-based questions and statements. Research has shown that the counselor's belief in the client's ability to change can be a significant determinant of outcome [17].

The dietitian's responsibility in counseling clients is to help reduce the barriers to making changes, focus more on the benefits, recognize and elicit "change talk," simplify the "to do" steps, and enhance self-efficacy. Some questions that might elicit change talk are as follows:

What do you think that you would like to do?
What options would you consider trying?
What would you say is the first step that you would like to take?

Once the dietitian has completed this process, it is the client's responsibility and choice to make a decision about changing eating habits.

According to Miller and Rollnick [17], motivation can be thought of not as a client's trait but as an interpersonal process between nutrition counselor and participant. Rather than seeing motivational change as something the

client achieves, this process is one that the nutrition counselor and the client experience in tandem. Use of this interactive process is of great value in achieving maintenance to goals in clinical trial research.

IV THEORIES USED IN ACHIEVING DIETARY BEHAVIOR CHANGE IN CLINICAL TRIALS

The nutrition components of clinical trials require skills in long-term dietary maintenance. These skills go beyond educating participants and instead involve strategies designed to reinitiate participants who no longer comply with the recommended eating plan. The studies described here provide research data collected when the theories presented previously are initiated in a clinical trial setting.

A Women's Health Initiative

The Women's Health Initiative (WHI) [18–20] is a randomized controlled clinical trial designed to look at prevention of breast and colorectal cancer. The dietary arm of this study focused on a diet with 20% energy from fat plus five servings of fruits and vegetables and six servings of grain products per day.

To accomplish this change in dietary habits, nutritionists in the study used a variety of behavior change techniques based on the models discussed here. The stages of change model drove efforts to increase compliance in the WHI. The Prochaska–DiClemente model includes six designated stages of change: precontemplation, contemplation, determination, action, maintenance, and relapse [21]. In an effort to simplify and accommodate different levels of adherence, WHI investigators chose to use only three levels of readiness to change: ready to change, unsure, and not ready to change. The decision to simplify levels is based on work with study participants showing that strategies to modify behavior fall within these three categories.

To test the effectiveness of using motivational strategies targeted at these three levels of change, a small research study was devised. Results of that study showed a positive change in dietary behavior following its implementation [22]. In this pilot study, researchers evaluated an intensive intervention program with diet. The basis of the program was the use of MI with participants in the WHI. The goal was to meet the study nutrition goal of 20% energy from fat.

WHI dietary intervention participants ($n = 175$) from three clinical centers were randomized to intervention or control status. Those randomized to the intensive intervention program participated in three individual MI contacts from a nutritionist, plus the usual WHI dietary intervention. Those randomized to the control group continued with the usual WHI dietary management intervention. Percentage energy from fat was estimated at intensive intervention program baseline and intensive intervention program follow-up (1 year later) using the WHI food frequency questionnaire (FFQ).

The change in percentage energy from fat between the intensive intervention program and the control group at baseline and 1-year follow-up was −1.2% points from the total fat for intensive intervention program participants and +1.4% points for control participants. The result was an overall difference of 2.6% points ($p < 0.001$).

Table 10.1 presents summary statistics on the intensive intervention program effects comparing baseline levels of fat consumption. The changes in fat consumed varied by intensive intervention program baseline fat intake as a percentage of energy intake. Participants having the highest intensive intervention program baseline fat intake (>30% energy) showed the largest overall change in percentage energy from fat between intensive intervention program baseline and intensive intervention program follow-up. As might be expected, the smallest change was found in participants who consumed between 25% and 30% of energy from fat at intensive intervention program baseline. These participants were closer to their goal of 20% energy from fat at baseline, allowing for less overall change.

The results of this study show that a protocol based on MI and delivered through contacts with trained dietitians is effective. Those subjects who participated in the intervention arm of the study further lowered their dietary fat intake to achieve study goals.

B The Diet Intervention Study in Children

A similar protocol was used in the Diet Intervention Study in Children (DISC) [23]. DISC was a randomized, multicenter clinical trial assessing the efficacy and safety of lowering dietary fat to decrease low-density lipoprotein cholesterol in children at high risk for cardiovascular disease [24,25]. Children began this study between ages 7 and 10 years and participated in group dietary intervention programs. As they moved into adolescence (ages 13–17 years) and encountered added obstacles to dietary adherence and retention, researchers in the study designed and implemented an individual-level motivational intervention. The diet prescription in the DISC study required providing 28% energy from total fat, less than 8% energy from saturated fat, up to 9% energy from polyunsaturated fat, and less than 75 mg/day of cholesterol. The diet met the age- and sex-specific Recommended Dietary Allowance for energy, protein, and micronutrients.

Researchers used a pre- to postintervention design among a subset of the total intervention cohort ($n = 334$).

TABLE 10.1 Effect of WHI (Pilot) Intervention on FFQ Percentage Energy from Fat Stratified by Baseline Percentage Energy from Fat[a]

	n	Baseline X (SD)	Follow-up X (SD)	Difference
% Energy from Fat <20.0				
Intervention control	23	17.75 (1.8)	17.86 (3.9)	0.1
	25	17.35 (2.3)	19.70 (4.5)	2.3
Difference[b]		−0.4	1.8	2.2
% Energy from Fat = 20.0 and <25.0				
Intervention control	25	22.72 (1.4)	21.68 (4.6)	−1.0
	26	23.17 (1.7)	25.29 (4.8)	2.1
Difference[b]		0.5	3.6	3.1
% Energy from Fat = 25.0 and <30.0				
Intervention control	21	27.42 (1.6)	26.3 (4.6)	−1.1
	15	26.94 (1.2)	26.89 (4.6)	0.0
Difference[b]		−0.5	0.6	1.1
% Energy from Fat = 30.0				
Intervention control	13	34.24 (2.5)	30.11 (6.5)	−4.1
	16	33.81 (3.1)	33.82 (5.0)	0.0
Difference[b]		−0.4	3.7	4.1

[a]*Participants with missing FFQ data were excluded.*
[b]$p < 0.05$ *using paired t-test.*
Source: Modified from D. Bowen, C. Ehret, M. Pedersen, L. Snetselaar, M. Johnson, Results of an adjunct dietary intervention program in the Women's Health Initiative, J. Am. Diet. Assoc. 102 (2002) 1631–1637.

The first 127 participants who appeared for regularly scheduled intervention visits after the implementation of the new intervention method were considered part of the study. These participants ranged from 13 to 17 years of age, with equal number of boys and girls. Nutrition interventionists asked all of the 127 participants to return in 4–8 weeks for a follow-up session. Initial sessions were conducted in person, and follow-up sessions were conducted either in person or by telephone.

Three 24-hour dietary recalls were collected within 2 weeks after the follow-up session. These dietary data were compared to three baseline 24-hour dietary recalls collected in the year preceding initial exposure to the motivational intervention method.

Self-reported data were also collected. At initial and follow-up intervention sessions, participants were shown "assessment rulers" (Fig. 10.1) numbered 1–12 and asked to rate their adherence to dietary guidelines and their readiness to make new or additional dietary changes.

Results from the study show that the mean energy from total fat decreased from 27.7% to 25.6% ($p < .001$) (Table 10.2) and the mean energy from saturated fat decreased from 9.5% to 8.6% of the total energy intake ($p < .001$). In addition, dietary cholesterol decreased from 182.9 to 157.3 mg ($p < .003$). A comparison of males and females showed no differences in gender relative to study results. Note that for this preliminary test, no control group was randomly assigned or examined. Therefore, the researchers cannot predict if significant reductions in consumption of dietary fat and cholesterol are attributable to the intervention.

The self-reported adherence rating score and readiness to change score increased by approximately 1 point on a scale from 1 to 12 (both $p < .001$). To help accomplish goals, action plans were also made. The study results show that 94% of the participants made action plans and 89% successfully implemented them.

This study also examined dietitian's satisfaction. The results showed that nearly three-fourths of the dietitians were satisfied or very satisfied with using the stages of change methods (Table 10.3).

C Motivational Intervention Method

DISC focused on the stages of change method with elements of MI. Fig. 10.2 provides a method for establishing

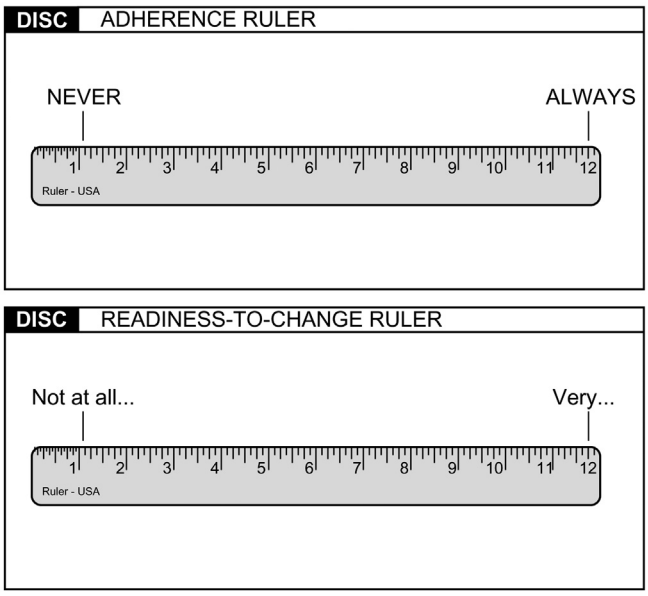

FIGURE 10.1 Assessment rulers. *Data from S.M. Berg-Smith, V.J. Stevens, K.M. Brown, L. VanHorn, N. Gernhofer, E. Peters, et al., for the Dietary Intervention Study in Children (DISC) Research Group, A brief motivational intervention to improve dietary adherence in adolescents, Health Educ. Res. 14 (1999) 399–410.*

TABLE 10.2 Changes in Total Fat Intake, Saturated Fat Intake, and Dietary Cholesterol After Two Intervention Sessions

	Mean	SD	*p*
Total Fat Intake			
Baseline	27.7	6.1	–
Follow-up	25.6	6.1	–
Change	−2.1	7.0	<0.001
Saturated Fat Intake			
Baseline	9.5	2.7	–
Follow-up	8.6	2.4	–
Change	−0.9	3.1	<0.001
Dietary Cholesterol			
Baseline	182.9	97.6	–
Follow-up	157.3	87.6	–
Change	−25.6	92.3	<0.003

Source: Data from S.M. Berg-Smith, V.J. Stevens, K.M. Brown, L. VanHorn, N. Gernhofer, E. Peters, et al., for the Dietary Intervention Study in Children (DISC) Research Group, A brief motivational intervention to improve dietary adherence in adolescents, Health Educ. Res. 14 (1999) 399–410.

TABLE 10.3 Nutrition Counselor Satisfaction with the Motivational Intervention Method

Level of Satisfaction	Percentage of the Intervention Sessions
Very satisfying	39
Satisfying	35
Somewhat satisfying	19
Slightly or not satisfying	7

Source: Data from S.M. Berg-Smith, V.J. Stevens, K.M. Brown, L. VanHorn, N. Gernhofer, E. Peters, et al., for the Dietary Intervention Study in Children (DISC) Research Group, A brief motivational intervention to improve dietary adherence in adolescents, Health Educ. Res. 14 (1999) 399–410.

rapport before tailoring the intervention to the readiness to change level: ready to change, unsure, and not ready to change. Fig. 10.3 provides specific strategies for each level of change.

1 First Level: Not Ready to Change

The main goal for this level of intervention is to raise awareness of the need to continue meeting goals (e.g., fat grams, carbohydrate grams, and energy intake). In addition, to achieve this goal, it is necessary to reduce resistance and barriers to meeting goals (e.g., decreasing cues to eat high-fat foods). Also, importantly, focus is placed on increasing interest in considering behavioral steps toward meeting the goals noted previously.

Throughout the initial interview, when working with a patient in this level, it is important to ask open-ended questions, listen reflectively, affirm, summarize, and elicit self-motivational statements. Fig. 10.3 provides examples of questions designed to facilitate the participant's ability to make motivational statements.

a Ask Open-Ended Questions

Initially for a participant at this level, it is important to ask questions that require explaining or discussing. Questions focus on requiring more than one-word answers.

The goal is to guide the participants to talk about their dietary change progress and difficulties. Fig. 10.2 provides some opening questions. Other questions and statements are presented here:

> "Let's discuss your experience with diet up to now."
> "Tell me how changing your diet has been for you."
> "What things would you like to discuss about your experiences with dietary change and your progress with changes? What do you like about these changes? What don't you like about these changes?"

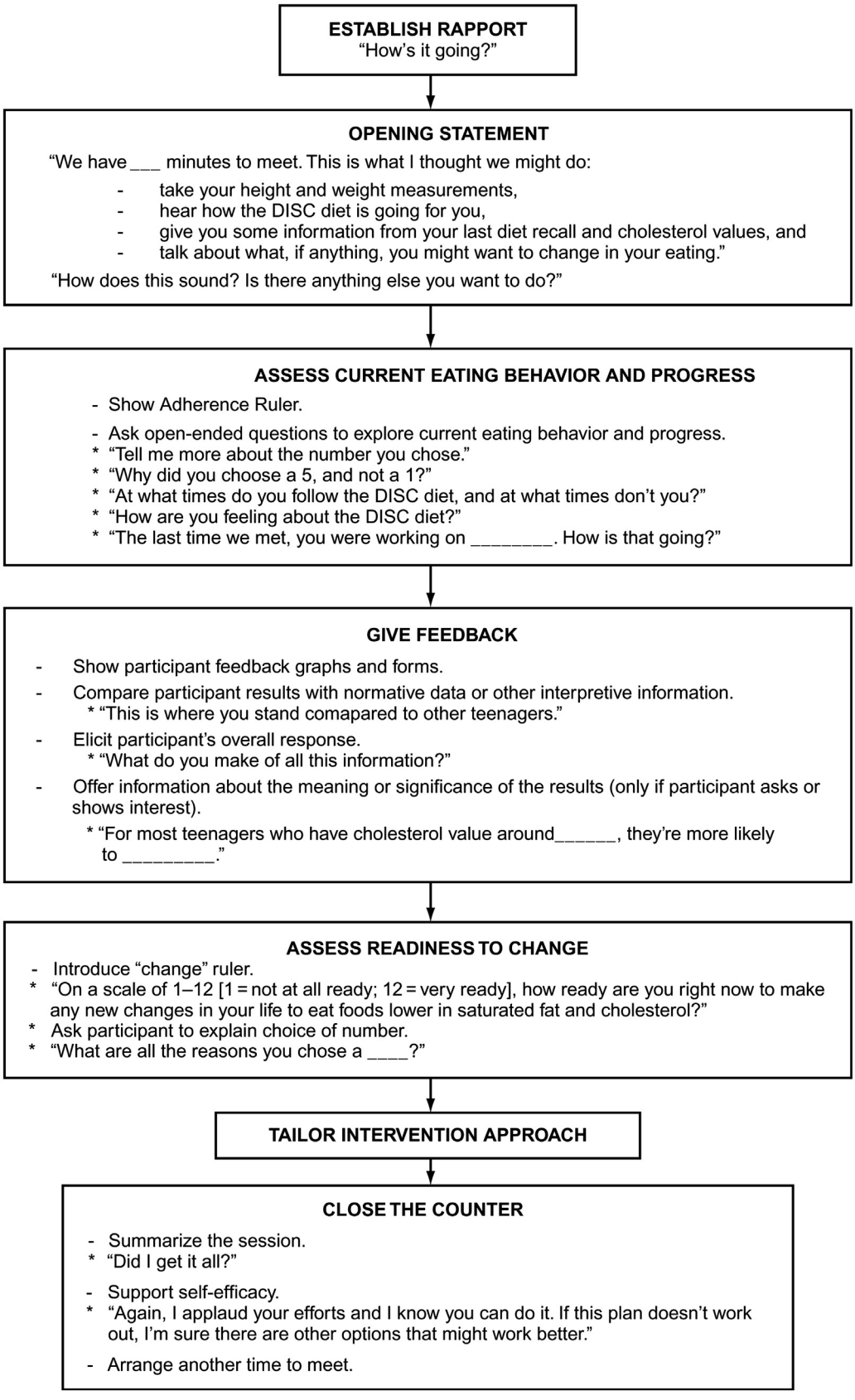

FIGURE 10.2 Stages of change model. *From S.M. Berg-Smith, V.J. Stevens, K.M. Brown, L. VanHorn, N. Gernhofer, E. Peters, et al., for the Dietary Intervention Study in Children (DISC) Research Group, A brief motivational intervention to improve dietary adherence in adolescents, Health Educ. Res. 14 (1999) 399–410.*

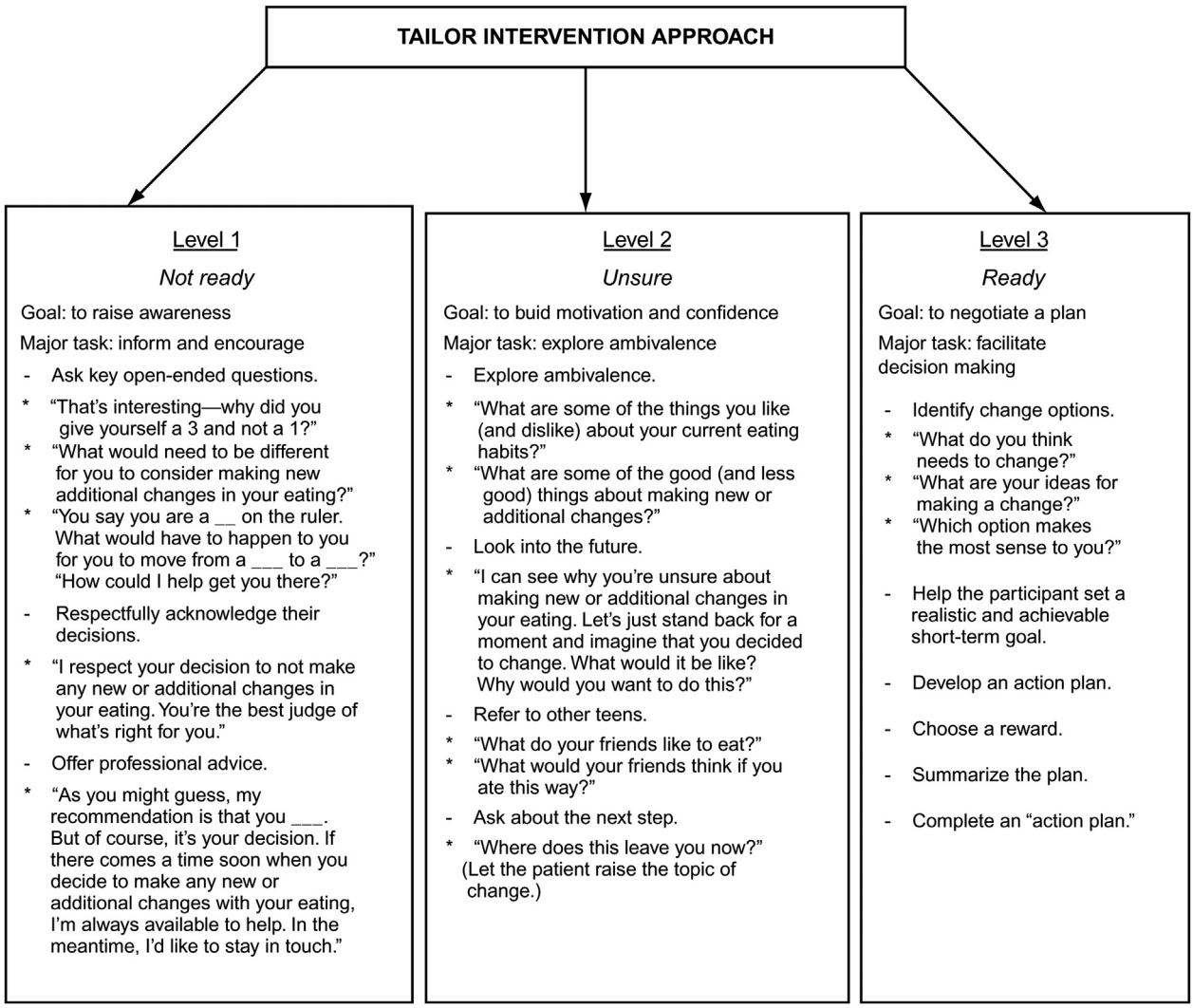

FIGURE 10.3 Stages of change components for three specific levels. *Modified from S.M. Berg-Smith, V.J. Stevens, K.M. Brown, L. VanHorn, N. Gernhofer, E. Peters, et al., for the Dietary Intervention Study in Children (DISC) Research Group, A brief motivational intervention to improve dietary adherence in adolescents, Health Educ. Res. 14 (1999) 399–410.*

b Listen Reflectively

Listening goes beyond hearing what a person has said and acknowledging those words. Crucial in responding to a patient or participant is the understanding of what is meant beyond the words. Reflective listening involves a guess at what the person feels and is phrased as a statement rather than a question. Stating the feeling behind the statement serves two purposes. It allows the participant to tell you if your judgment of the feeling is on target. It also shows that you really are trying to understand more than just words and do care about feelings also. The following are some participant–nutritionist interactions that illustrate reflective listening:

Scenario 1

Participant: "There are times when I do a wonderful job of meeting my fat gram goal, but sometimes I don't do so well. I keep trying though."

Dietitian: "You seem to feel badly that you don't always meet your fat gram goal."

Scenario 2

Participant: "I am so tired of trying to follow this diet. It seems that I have put hours into following it, and I have little to show for it. I certainly have not lost weight."

Dietitian: "You feel frustrated and angry about trying so hard and still getting nowhere."

Scenario 3

Participant: "When I don't fill in a food diary, I am not sure that I am doing well or not."

Dietitian: "You are worried on days when you do not fill in a food diary."

Scenario 4

Participant: "I really don't want to continue following this diet. I have other things that are more of a priority now."

Dietitian: "You seem hassled by these other competing desires and feel that following a new eating pattern is getting in the way."

c Affirm

Communicating support to participants is an excellent way of letting them know that you appreciate what they are doing. Affirmations are statements that indicate alignment and normalization of the participant's issues. Alignment means telling participants that you understand them and are with them in their difficulties. Normalization means telling the participants that they are perfectly within reason and "normal" to have such reactions and feelings. Examples of affirmations include the following:

"It is very hard to struggle with competing priorities. You've done amazingly well."

"That is an insightful idea."

"Thank you for telling me that. It must have been hard for you to tell me."

"I can see why you would have this difficulty. Many people have the same problem."

d Summarize

Periodically, and at the point when you begin to elicit self-motivational statements, summarize the content of what the participant has said. Cover key points even if they involve negative feelings. You can discuss conflicting ideas that the participant has brought up by using the strategy "on the one hand, you … and on the other hand, you …." This reminds both of you about the issues and ensures clarity.

e Elicit Change Statements

The most important part of self-motivational statements is that they help participants realize that a problem exists, that they are concerned about the problem, that they intend to correct the problem, and that they think they can do better in the future. Fig. 10.4 provides questions to

1. **Problem Recognition**
   - What things make you think that this is a problem?
   - What difficulties have you had in relation to your diet?
   - In what ways do you think you or other people have been inconvenienced by your diet?
   - In what ways has this been a problem for you?
   - How has your diet stopped you from doing what you want to do?

2. **Concern**
   - What is there about your diet that you or other people might see as reasons for concern?
   - What worries you about your diet?
   - How do you feel about your dietary problems?
   - How much does that concern you?
   - In what ways does this concern you?
   - What do you think will happen if you don't make a change?

3. **Intention to Change**
   - The fact that you're here indicates that at least a part of you thinks it's time to do something. What are the reasons you see for making a change?
   - What makes you think that you need to make a change?
   - If you were 100% successful and things worked out exactly as you would like, what would be different?
   - What things make you think that you should stop following your diet? . . . And what about the other side? What makes you think it's time for a change?
   - What are you thinking about your diet at this point?
   - What would be the advantages of making a change?
   - I can see that you're feeling stuck at the moment. What's going to have to change?

4. **Optimism**
   - What makes you think that if you decide to make a change, you could do it?
   - What encourages you that you can change if you want to?
   - What do you think would work for you, if you decided to change?

FIGURE 10.4 Examples of questions designed to elicit self-motivational statements. *Modified from D. Bowen, C. Ehret, M. Pedersen, L. Snetselaar, M. Johnson, Results of an adjunct dietary intervention program in the Women's Health Initiative, J. Am. Diet. Assoc. 102 (2002) 1631–1637.*

elicit self-motivational statements. These statements fall into four categories: problem recognition, concern, intention to change, and optimism. It is important to respectfully acknowledge decisions that participants make. These decisions may mean that a participant has decided not to make changes immediately (see Fig. 10.3). It is appropriate to offer professional advice but still leave the actual decision to make a change up to the participant. Fig. 10.3 provides some ideas on how to approach the participant. Close the discussion with another summary. Concentrate on any self-motivational statements that the participant has made. End the session with the idea that both of you should think about what has been discussed and that you can revisit the issues next time.

2 Second Level: Unsure About Change

The main goal for this intervention is to tip the balance toward working to meet the goals. Four steps are important in meeting the goals: regroup, ask key questions, negotiate a plan, and conclude the work.

a Regroup

The first step in dealing with a participant who is unsure about changing dietary habits is regrouping to focus on the transition from not being ready to deal with the problems of change to moving toward a reinitiating of behavior adjustment. This process of regrouping can serve as a reminder of what has happened in previous sessions. The following are four ways to regroup:

1. Summarize the participant's perceptions of what is going on. The summary might include self-motivational statements that the participant has made.
2. Identify ambivalence or other conflicting issues.
3. Review any self-monitoring related to dietary intake.
4. Restate intentions or plans to change or do better in the future.

b Ask Key Questions

Ask questions that focus on the participant's statements regarding future plans to make dietary changes. The goal is to ask open-ended questions that cause the participant to think about what you have just summarized and come to the conclusion that action is necessary. The goal is for the participant to provide a statement showing the desire to change. Here are some examples of questions that facilitate positive participant statements:

"How might we work together to proceed from here?"
"Hearing my summary of how things have gone in the past, what do you want to do?"
"How might you become more involved in dietary change?"
"What are the good parts and the bad parts about continuing to change?"
"You are currently unsure of what to do. How might we work together to resolve the issue?"

c Negotiate a Plan

There are three parts to the negotiation process. The first involves setting goals; the second, considering the options; and finally, arriving at a plan:

Set goals: Past wisdom dictated that setting goals meant being specific and behavioral. "I will eat candy bars only one time per week, on Sunday." MI dictates that goal setting may start out broadly at first and then move to behavioral goals that are specific. To elicit broadly stated goals, the following questions might be used:

"What about your diet would you like to change?"
"How would you like things to be different from how they are now?"

Consider options: Make a list of things that might be changed to bring the participant closer to the dietary goal. Ask the participant to choose among the options. If the first one does not work, the participant has many choices as backups.

Arrive at a plan: Ask the participant to arrive at a plan. Include in the plan the specific behavioral goals with potential problems that may serve as barriers to making these changes.

d Conclude the Work

Always end the session with an encouraging statement and a reflection on the participant's resourcefulness in identifying the plan. Follow this statement with the idea that he or she is the best expert relative to behavior change. Indicate that you will stay in touch to check on how the behavior change is going.

3 Third Level: Ready to Change

The main goal for this third intervention is to reengage the participant in meeting the dietary goals.

a Review

Cover the previous discussions with the participant. Focus on the statements the participant made that show interest and a willingness to change. Use statements that show that it was the participant's idea to meet dietary goals previously set—e.g., "You said that you were interested in trying again."

b Encourage Choices and Activities

Ask the participant what he or she would like to do to reengage. Encourage the participant to make his or her

own choice. Collaborate and negotiate short-term, easily attained goals initially, such as "I will drink 1% milk for a week in place of 2% milk and gradually reduce to fat-free milk."

c Summarize

Review the discussion, the issues, and the difficulties on both sides. Remind the participant to keep trying and to believe in himself or herself.

D The Modification of Diet and Renal Disease Study

The Modification of Diet and Renal Disease Study (MDRD) [26] used the self-management approach to modify dietary behavior. In this population of persons diagnosed with renal disease, research nutritionists counseled participants on diets low in protein and phosphorus. Although the diets were difficult to follow, the study participants showed great motivation based on their desires to avoid renal dialysis [27]. The following strategies were used successfully in the MDRD [28]:

Single-nutrient approach to dietary behavior change: The focus in this study was on reducing protein content of the diet. With this reduction, dietary phosphorus was also reduced. When other nutrients were modified, specific food groups were identified. Even with changes in other nutrients, protein was still a primary focus.

Self-monitoring: The participant's ability to self-monitor was crucial to keeping protein intake down to goal levels. Weighing and measuring was used as a means of matching dietary change on a daily basis with the biological marker of urinary nitrogen. Further study in self-monitoring matched with the biological marker showed that problems occur in knowing how to best represent dietary intake [29]. For example, it is often difficult to closely mirror the exact amount of protein in a cut of meat if that cut is not precisely specified.

Staging changes: In the MDRD, dietitians also staged changes in dietary protein by gradually reducing the dietary protein intake. This gradual change made day-to-day modifications easier.

Modeling: Dietitians modeled dietary changes by offering both recipes low in protein and taste-testing sessions. Group sessions were held at which a special meal was offered with food preparation techniques modeled.

E The Diabetes Control and Complications Trial

The Diabetes Control and Complications Trial (DCCT) [30] used techniques similar to those of the MDRD study [31]:

Single-nutrient approach: Investigators focused on carbohydrate as a single nutrient, where it was matched with insulin to achieve normalized blood glucose.

Self-monitoring: Monitoring consisted of following blood glucose concentrations and dietary intakes to verify where problems might be occurring. If dietary intake was high along with blood glucose concentrations, dietary intake or insulin was modified to achieve normal blood glucose levels.

Staging changes: Changes were staged by working on specific times of day that were most problematic. If lunchtimes were most often high, we focused on dietary intake modifications to alter blood glucose levels. Also, insulin and exercise often played a role.

Modeling: Dietary modifications were facilitated by providing recipes, modeling, and going to restaurants to identify and anticipate blood glucose levels after eating a favorite lunch or other meals out of the home.

F The Brief Motivational Intervention to Reduce Body Mass Index Study

The Brief Motivational Intervention to Reduce Body Mass Index (BMI^2) study is designed to compare three groups: pediatricians without MI training, pediatricians with MI training, and pediatricians and registered dietitians with MI training. The outcome of this study is children with elevated BMIs ranging from the 85th to the 97th percentile for their ages. Clinicians in this study will work with parents to try to modify their way of working with children to achieve dietary behavior change. The basis of this work is the self-determination theory, which serves as the underlying construct for the MI communication style. This is a 2-year intervention that is currently in its first year of data collection [32]. The study showed that the use of MI in the combined group of dietitians and pediatricians resulted in greater reductions in children's BMI toward healthy levels [33].

V SUMMARY

Considerable experience in clinical trials suggests that dietary modification requires a process of making changes on an individual basis with constant negotiation with the client or participant. Working as a team, the nutritionist and participant can achieve dietary change that alters biological markers, may reduce disease risk, and optimizes management.

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Chapter 11

Biomarkers and Their Use in Nutrition Intervention*

Amanda J. Cross[1], Johanna W. Lampe[2], Cheryl L. Rock[3] and Carol J. Boushey[4]

[1]Imperial College, London, United Kingdom, [2]Fred Hutchinson Cancer Research Center, Seattle, WA, United States, [3]University of California at San Diego, La Jolla, CA, United States, [4]University of Hawaii Cancer Center, Honolulu, HI, United States

I INTRODUCTION

A biomarker or biological indicator is a characteristic that is measured and evaluated as a marker of normal biological processes, pathogenic processes, or responses to an intervention. In theory, almost any measurement that reflects a change in a biochemical process, structure, or function can be used as a biomarker. In addition, an exogenous compound that, as a result of ingestion, inhalation, or absorption, can be measured in tissues or body fluids can also be considered a biomarker.

Biomarkers can be classified broadly into markers of exposure, effect, and susceptibility and have numerous applications in nutrition. They can be used to assess dietary intakes (exposure), biochemical or physiological responses to a dietary behavior or nutrition intervention (effect), a clinical end point or disease outcome (surrogate end point biomarker), and predisposition to a disease or response to treatment (genetic susceptibility).

Although clinicians have used certain biological markers, such as serum cholesterol and glucose, for generations, the use of biomarkers has taken on new importance with the dramatic advances in various fields of biology and desire for objective measures in large-scale, population-based, descriptive, and intervention nutrition research. Exquisitely sensitive laboratory techniques can detect subtle alterations in molecular processes that reflect events known or believed to occur along the continuum between health and disease.

This chapter presents the basic concepts and key issues related to the various uses of biomarkers in nutrition intervention research; it is not intended to be a comprehensive review. Identification and use of biomarkers is continuously evolving with the growing understanding of biological processes and the improved sensitivity of laboratory assays. Consequently, our examples of existing biomarkers are snapshots of the greater scheme of biomarker development and application.

II BIOMARKERS OF DIETARY INTAKE OR EXPOSURE

Biomarkers are used to monitor dietary exposure and for nutritional assessment for several reasons. One reason is to provide biochemical data on nutritional status by generating objective evidence that enables the evaluation of dietary adequacy or ranking of individuals on exposure to particular nutrients or dietary constituents. Biochemical or biological measurements may also be collected to characterize objectively a dietary pattern, such as fruit and vegetable consumption; to validate dietary assessment instruments or self-reported dietary data; or to monitor compliance to a dietary intervention. Another purpose for obtaining these biological measures is to establish the biological link between the nutritional factor and a physiological or biochemical process—often a hallmark of nutrition intervention studies—when the concentration of the micronutrient or dietary constituent is measured in a peripheral tissue.

Biomarkers of dietary intake can be classified as either recovery biomarkers, which are considered the best, or concentration biomarkers. Recovery biomarkers reflect absolute

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intake over a defined period of time; an example is nitrogen in 24-hour urine collections as a recovery biomarker of dietary protein intake [1]. Concentration biomarkers, on the other hand, are correlated with intake but cannot be used to calculate an absolute level of intake; an example of a concentration biomarker is plasma vitamin E [2].

A Biomarkers of Energy Intake

To date, few biological measures are available that objectively monitor overall energy intake, and those that are available are cumbersome in community dwelling populations or expensive. Under steady-state conditions, indirect calorimetry provides an estimate of energy expenditure and some insight about intake. Indirect calorimetry estimates the rate of oxidation or energy expenditure from the rate of oxygen consumption (VO_2) and the rate of carbon dioxide production (VCO_2). This technique is relatively inexpensive and portable, although some participant's cooperation is required. These traits make the technique attractive primarily for clinical applications [3].

Energy expenditure can also be measured using a doubly labeled water technique [4], as discussed in detail in Chapter 4, Energy Requirement Methodology, which is an example of a recovery biomarker in which energy intake is indirectly ascertained by measuring energy expenditure. This method uses nonradioactive isotopes of hydrogen (^2H) and oxygen (^{18}O) to measure community dwelling total energy expenditure by monitoring urinary isotope excretion. Energy expenditures determined by room calorimetry, indirect calorimetry, and doubly labeled water measures are not significantly different within the calorimeter environment; however, in community dwelling individuals, doubly labeled water-derived energy expenditures are found to be 13−15% higher than those for other methods [5]. The doubly labeled water method has the distinct advantage of allowing the study participants to go about their usual activities, with energy expenditure calculated after a study period of 7−14 days. Unfortunately, the ^{18}O isotope required to conduct doubly labeled water studies is expensive and is often in short supply. Although doubly labeled water methodology is suited to nutrition research aimed at quantifying total energy expenditure for specific groups, the cost of large samples limits broad use.

B Biomarkers of Nutrient Intake

Biochemical measures of nutrients can be a valuable component of nutritional assessment and monitoring. Overall, the usefulness of biochemical indicators of nutritional status or exposure is based on knowledge of the physiological and other determinants of the measure. For several micronutrients, the concentration of the nutrient in the circulating body pool (i.e., serum) appears to be a reasonably accurate reflection of overall status for the nutrient. In contrast, the amount of some micronutrients in the circulating pool may be homeostatically regulated when the storage pool is adequate, or may be unrelated to intake, and thus has little relationship with total body reserves or overall status. Fig. 11.1 illustrates the relation between various compartments or body pools that may be sampled in the measurement of biological indicators.

Knowledge of the influencing nondietary factors is particularly important for accurate interpretation of the nutrient concentration in tissues. For example, tocopherols and carotenoids are transported in the circulation nonspecifically by the cholesterol-rich lipoproteins [6,7], so higher concentrations of these lipoproteins are predictive of higher concentrations of the associated micronutrients in the circulation, independent of dietary intake or total body pool. Smoking and alcohol consumption need to be considered in the interpretation of serum and other tissue concentrations of several micronutrients, particularly compounds that may be subject to oxidation (e.g., vitamin C, tocopherols, carotenoids, folate). Knowledge of the relationship between the indicator and the risk of nutrient depletion, in addition to the responsiveness of the indicator to interventions or change, is also necessary [8]. For some nutrients, a specific sensitive exposure marker of diet has not yet been identified.

Table 11.1 lists examples of biochemical measures of nutrients that may serve as useful biomarkers in nutritional assessment or monitoring of dietary intake; all of these are examples of concentration biomarkers and may not be proportionately related to intake because their levels are the result of other influencing factors. For more details, the reader is referred to in-depth reviews addressing the use of biomarkers for assessing nutrient exposure [9−12].

C Biomarkers of Other Dietary Exposures

Numerous dietary constituents, particularly of plant origin, although not recognized as essential for life, have demonstrated biological activity and are thought to play an important role in the prevention of chronic diseases [13,14]. These phytochemicals are absorbed to various degrees, often metabolized in the intestinal epithelium and liver, and excreted; thus, the metabolites can be monitored in serum or plasma or urine.

Some classes of compounds such as flavonoids are found in many plant foods, whereas others such as isoflavones are limited to select sources (Table 11.2). The isoflavones daidzein and genistein are highly concentrated in soybeans and soy products [15,16]. Urinary isoflavone excretion is associated strongly and directly with soy protein intake under controlled dietary conditions [17]. In intervention studies of soy foods or isoflavone supplementation,

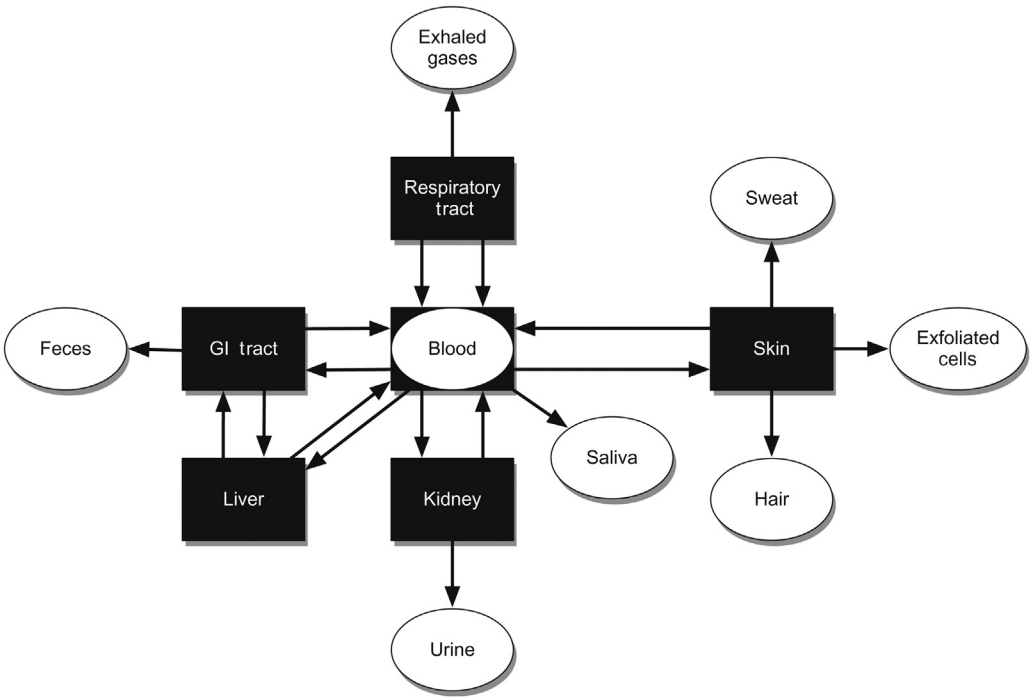

FIGURE 11.1 The relation between body compartments and biological specimens that can be assayed for dietary biomarkers.

urinary isoflavonoid excretion and serum or plasma isoflavone concentrations are useful markers of study compliance [18,19]. However, as the plasma half-lives of the isoflavones genistein and daidzein are short (6–8 hours) [20], the timing of soy consumption in relation to urine or blood sampling may under- or overestimate isoflavone exposure. Metabolism of isoflavones is also linked to the health of colonic bacterial populations, and therefore the effects of diet and drugs on the colonic environment may influence plasma and urinary levels.

Dietary exposure to flavonoids and other polyphenols can be monitored by measuring parent compounds and metabolites in urine or plasma [21,22]. Several compounds in cruciferous vegetables, such as sulforaphane and other isothiocyanates, have been of interest because of their potential chemopreventive effects. Concentrations of these compounds and their metabolic derivatives can be measured in plasma and urine by liquid chromatography–mass spectrometry (LC-MS) [23]. In addition, dithiocarbamates (conversion products of isothiocyanates and their metabolites) can be quantified readily in urine, following extraction and measurement by high-performance liquid chromatography (HPLC). Both these approaches provide a way to monitor cruciferous-vegetable exposure during an intervention [24].

Biomarkers also exist for monitoring exposure to less desirable food constituents, such as mycotoxins (e.g., aflatoxin) in mold-contaminated grain products and pyrolysis products that result from cooking meat at high temperatures (e.g., heterocyclic amines). Because of the nature of these compounds, exposure to potentially carcinogenic compounds can be determined by measuring the presence of adducts—the result of covalent binding of the chemicals to proteins or to nucleic acids in DNA. The rationale for using measurements of carcinogen–DNA adducts is based on the assumption that DNA adducts formed in vivo are responsible for genetic alterations in genes critical for carcinogenesis and that protein adducts formed through the same processes reflect the formation of DNA adducts [25]. Because adducts represent an integration of exposure and interindividual variability in carcinogen metabolism and DNA repair, they may provide a more relevant measure of exposure (i.e., a biologically effective dose [25]). Some adducts are specific for dietary exposure; aflatoxin–albumin adducts result from ingestion of aflatoxin. Other adducts, such as benzo[a]pyrene–DNA adducts, are nonspecific because benzo[a]pyrene comes from a variety of sources besides diet, including air pollution, tobacco, and occupational exposures. Adducts can be used to monitor exposure within individuals. They can also serve as early markers of the efficacy of interventions designed to prevent exposure to genotoxic agents or to modify the metabolism of procarcinogens once exposure has occurred. An example of this latter use is an intervention to reduce aflatoxin–DNA adducts using a broccoli sprout supplement [26].

TABLE 11.1 Biomarkers of Nutrient Intake

Nutrient	Biomarkers of Dietary Exposure	Possible Functional Markers
Dietary fiber, nonstarch polysaccharides	Fecal hemicellulose	Fecal weight
		Fecal short-chain fatty acids
	Urinary or plasma lignans	
	Urinary or plasma alkylresorcinols (whole grains)	
Thiamin		Erythrocyte transketolase activation
Biotin	Urinary 3-hydroxy-isovalerate with a loading dose of leucine	Erythrocyte pyruvate carboxylase activity
Riboflavin	Plasma FAD	EGRAC
	Erythrocyte FAD	
Niacin	Erythrocyte NAD	Erythrocyte nicotinate-nucleotide: pyrophosphate phosphoribosyltransferase activity (not very responsive)
	Urinary metabolites of niacin	
Vitamin B_6	Plasma pyridoxal 5-phosphate	Erythrocyte aspartate or alanine aminotransferase
	Urinary 4-pyridoxic acid	
Folate	Plasma folate	Plasma homocysteine
	Erythrocyte folate	
Vitamin B_{12}	Plasma B_{12}	
Vitamin C	Plasma vitamin C	Urinary deoxypyridinoline:total collagen cross-links
	Erythrocyte, lymphocyte, or platelet vitamin C	Urinary carnitine
Vitamin A	Plasma retinol:retinol-binding protein	
Vitamin E	α- and/or γ-tocopherol in serum or plasma, erythrocytes, lymphocytes, lipoproteins, adipose tissue, or buccal mucosal cells	LDL oxidation
		Breath pentane and ethane
		Platelet adhesion and aggregation
Vitamin D	Serum 25-hydroxyvitamin D	
Vitamin K	Plasma vitamin K	Plasma prothrombin concentrations
Phosphorus	Serum inorganic phosphate	
Magnesium	Erythrocyte or lymphocyte magnesium	
Calcium	Calcium retention	Bone mass
		Serum osteocalcin
		Serum levels of skeletal alkaline phosphatase
		Urinary and serum measures of collagen turnover
Iron		Serum ferritin[a]
		Transferrin saturation
		Erythrocyte protoporphyrin

(*Continued*)

TABLE 11.1 (Continued)

Nutrient	Biomarkers of Dietary Exposure	Possible Functional Markers
		Mean corpuscular volume
		Serum transferrin receptor
		Hemoglobin or packed cell volume
Copper	Platelet copper	Erythrocyte SOD
		Platelet cytochrome *c* oxidase activity
		Serum peptidylglycine α-aminating monooxygenase activity
		Plasma diamine oxidase
Zinc		Erythrocyte metallothionein
		Erythrocyte SOD
		Monocyte metallothionein mRNA
		Serum thymulin activity
		Plasma 5-nucleotidase activity
Manganese	Serum manganese	Lymphocyte Mn-SOD activity
		Blood arginase activity
Molybdenum		Urinary levels of sulfate, uric acid, sulfite, hypoxanthine, xanthine, and other sulfur-containing compounds
Iodine	Urinary or plasma iodine	Plasma TSH, T_4, and T_3 (total and free)
Selenium	Plasma or whole-blood selenium	Plasma GSH peroxidase activities
	Hair or toenail selenium	Erythrocyte GSH peroxidase activities
		Blood cell selenoperoxidase activities

[a]*In approximate order of increasing severity of iron shortage [109].*
EGRAC, FAD-dependent erythrocyte glutathione reductase activation coefficient; *FAD,* flavin adenine dinucleotide; *GSH,* glutathione; *LDL,* low-density lipoprotein; *NAD,* nicotinamide adenine dinucleotide; *SOD,* superoxide dismutase; *T_3,* triiodothyronine; *T_4,* thyroxine; *TSH,* thyroid-stimulating hormone.
Direct measures of dietary exposure and nutrient-specific functional markers. This table includes both established markers and additional markers that show promise.

D Biomarkers as General Dietary Indicators

Although biomarkers of energy intake, specific nutrients, and other dietary variables can themselves be useful indicators of general diet, there are additional broad biomarkers to consider. Monitoring changes in patterns in response to dietary interventions presents additional challenges. The goal in this case is to monitor the intake of certain types of foods or food groups rather than specific nutrients; therefore, these dietary indicators should ideally be distributed within certain types of foods.

Plasma carotenoids provide a good example of the use of biomarkers as a dietary indicator when the goal is to assess and monitor dietary patterns. Vegetables and fruits contribute the vast majority of carotenoids in the diet, and plasma carotenoid concentrations have been shown to be useful biomarkers of vegetable and fruit intakes in cross-sectional descriptive studies, controlled feeding studies, and clinical trials [27–30]. The consistency of this relationship across diverse groups and involving various concurrent dietary manipulations (with differences in amounts of dietary factors that could alter carotenoid bioavailability) is notable, although considerable interindividual variation in the degree of response is typically observed. Also, nondietary factors that are among the determinants of plasma carotenoid concentrations (e.g., body mass and plasma cholesterol concentration) will influence the absolute concentration that is observed in response to dietary intake, so these characteristics must be used as adjustment factors.

Although vitamin C also is provided predominantly by fruits and vegetables in the diet, this measure is much less useful as a biomarker of this dietary pattern because the relationship between vitamin C intake and plasma

TABLE 11.2 Phytochemical Content of Plant Food Families and Select Plant Foods

Plant Foods	Flavonoids	Isoflavones	Lignans	Carotenoids	Organosulfides	Isothiocyanates	Terpenes	Phytates
Cruciferae[a]	✓			✓	✓	✓	✓	
Rutaceae[b]	✓			✓			✓	
Alliaceae[c]	✓			✓	✓		✓	
Solanaceae[d]	✓			✓			✓	
Umbelliferae[e]	✓			✓			✓	
Curcurbitaceae[f]	✓			✓			✓	
Cereals	✓		✓				✓	✓
Soybeans	✓	✓		✓			✓	✓
Flaxseed	✓		✓					
Measurable in biological samples	Urine	Urine	Blood	Blood	Blood	Urine	Blood	Urine
Blood	Blood			Breath	Blood	Stool	Blood	
	Stool	Stool						

[a]*Cabbage family.*
[b]*Citrus family.*
[c]*Onion family.*
[d]*Tomato family.*
[e]*Carrot family.*
[f]*Squash family.*
Some phytochemicals are present in most plant foods; others are restricted to particular botanical families or even particular plant species.
Source: Adapted from A.B. Caragay, Cancer-preventive foods and ingredients. Food Technol. 46 (1992) 65–68 and J.W. Fahey, B.A. Clevidence, R.M. Russell, Methods for assessing the biological effects of specific plant components. Nutr. Rev. 57 (1999) S34–S40.

concentration is linear only up to a certain threshold [31]. The use of vitamin C supplements (which is common in the US population) often increases the intake level beyond the range in which linearity between intake and plasma concentration occurs and thus obscures the relationship between food choices and tissue concentrations.

Lignans are a group of compounds present in high-fiber foods, particularly cereals and fruits and vegetables [32]. These compounds are not found in animal products and, similar to carotenoids, may be useful markers of a plant-based diet [33]. Lignans provide an example of how using dietary constituents as biomarkers requires an understanding of the metabolism of the compounds. Lignans in plant foods are altered by intestinal microbiota so that the specific compounds, enterodiol and enterolactone, monitored in plasma or urine are actually bacterial metabolites. Because of this bacterial conversion, lignan concentrations in urine or plasma in response to a similar dietary dose vary significantly among individuals. In addition, nondietary factors (e.g., use of oral antibiotics) reduce enterolactone and enterodiol production [34].

Although whole grain foods are a source of fiber, they have independently been inversely associated with some chronic diseases, such as cardiovascular disease [35], type 2 diabetes [36], and some cancers [37,38]. The accurate assessment of whole grains from standard dietary questionnaires is challenging; therefore, a biomarker of intake is essential. Plasma concentrations and urinary excretion of alkylresorcinols and alkylresorcinol metabolites have been identified specifically as good indicators of whole grains intake[39,40].

In contrast to fiber, red and processed meats have been positively associated with stroke, cardiovascular disease, diabetes, and cancer [41–46]; these associations were identified using self-reported dietary assessment methods known to result in measurement error. Two controlled feeding studies were used to investigate potential biomarkers of meat intake by analyzing known breakdown products, including creatinine, creatine, carnitine, taurine, 1-methylhistidine, and 3-methylhistidine. Cross and colleagues reported that urinary levels of 1-methylhistidine and 3-methylhistidine dose-dependently reflected red meat intake [47]. After combining the self-reported meat intake data with the urinary data, it was found that there were no associations between 1- and 3-methylhistidine and colorectal adenoma [48]. Aside from urine analysis, hair is also a possible biomarker of meat intake. A carcinogenic substance of cooked meat, 2-amino-1-methyl-6-phenylimidizo [4,5-b]pyridine, has been shown to be measured in naturally colored hair and dyed hair [49]. However, this needs to be investigated in community dwelling individuals, in whom diet is not tightly controlled, to determine whether these can be successfully used in an epidemiologic setting as potential biomarkers of meat intake.

Investigating specific fatty acids can reveal important dietary patterns. The fatty acid composition of membrane phospholipids is in part determined by the omega-6 and omega-3 fatty acid composition of the diet. Thus, the fatty acid pattern of serum phospholipids or plasma aliquots has been used as a biomarker of compliance with omega-3 fatty acid supplementation in clinical trials [50,51]. Although enzyme selectivity and other physiological factors are also important determinants of the fatty acid composition of phospholipids, a diet high in omega-3 polyunsaturated fats will result in increased amounts of eicosapentaenoic and docosahexaenoic acids in circulating tissue pools. Specific fatty acids can also be associated with certain types of foods. Pentadecanoic acid (15:0) and heptadecanoic acid (17:0) are fatty acids produced by bacteria in the rumen of ruminant animals. These fatty acids, with uneven numbers of carbon atoms, are not synthesized by humans; therefore, their presence in human biological samples can indicate dietary exposure to milk fat. Proportions of 15:0 and 17:0 in adipose tissue and concentrations of 15:0 in serum have been found to correlate with milk fat intake in men and women [52,53].

III FUNCTIONAL BIOMARKERS AND MARKERS OF BIOLOGICAL EFFECTS

If a nutrient or dietary constituent has an identified impact on physiological, biochemical, or genetic factors, measuring markers of those effects can be extremely useful. Such indices can be classified as those that are measures of discrete functions of a nutritional factor and those that are measures of more general functions or activities [54]. A discrete functional index often relates to the first limiting biochemical system—e.g., a particular enzymatic pathway [55]. These markers can be used to identify the dosage or concentration of a nutritional factor necessary to achieve a clinically meaningful response or to define optimum nutrient status (Table 11.1). Unfortunately, for many nutritional factors, the first limiting biochemical system is unknown or not readily measured or accessible in humans. A general functional index is less specific but may be more directly linked to the pathogenesis of disease or ill health. Often, a panel of markers, rather than one specific measure, provides a better picture. Examples of general functional indices or markers are oxidative stress, immune function, bone health, and cell turnover—processes that have been shown to play roles in the risk of various diseases.

For a functional index to be an effective nutritional biomarker for intervention studies related to disease risk, a cause-and-effect relationship must be established

(1) between nutritional status and the functional index, (2) between the functional index and ill health, and (3) between nutrient status and ill health. Such an undertaking is a daunting and time-consuming task, but it is especially important if a functional biomarker is going to be used as a proxy, or surrogate, for a clinical end point or disease outcome. A clinical end point is a characteristic or variable that measures how a patient feels, functions, or survives. A surrogate end point biomarker is an index whose modulation has been shown to indicate the progression or reversal of the disease process; it is a biomarker that is intended to substitute for a clinical end point. In an intervention trial, the use of surrogate end point biomarkers (rather than the frank diagnosis of disease) requires substantially less time and fewer resources in the evaluation of efforts aimed toward reducing risk for chronic diseases such as cancer, cardiovascular disease, and osteoporosis [56].

To date, few markers have been established as true surrogate end point biomarkers (i.e., they can accurately substitute for a clinical end point [57]). The evidence supporting the linkage of a biomarker to a clinical end point may be derived from epidemiological studies, clinical trials, in vitro analyses, animal models, and simulated biological systems. Many biomarkers have been proposed as potential surrogate end points, but relatively few are likely to achieve this status because of the complexity of disease mechanisms and the limited capability of a single biomarker to reflect the collective impact of multiple therapeutic effects on ultimate outcome.

A Biomarkers of Enzyme Function

Understanding how diet influences enzyme systems is important in developing strategies for disease prevention and treatment. For example, dietary modulation of enzymes involved in carcinogen metabolism may be important in reducing cancer risk, and a dietary intervention that reduces expression of rate-limiting enzymes in cholesterol synthesis may alter cardiovascular disease risk. Enzymes that require micronutrients as cofactors are also used as biomarkers of nutritional status (Table 11.1).

Components of the diet have the capacity to modulate protein synthesis and function. An ideal discrete functional marker would be one that reflects the direct effect of a dietary constituent—e.g., mRNA amount when the dietary factor regulates gene expression or level of enzyme activity when the factor acts as a competitive inhibitor of the enzyme (Fig. 11.2). Unfortunately, monitoring at these levels in the pathway in an intact human is not always feasible. Often, we rely primarily on a downstream marker, whose measurement may be influenced by subsequent or parallel pathways and may give a diluted signal.

Often, the enzymes of interest are located primarily in tissues that are not readily accessible (e.g., liver, intestine, and lung). One approach to meeting this challenge is to measure the enzymes in more accessible tissue; e.g., enzymes that are present in high levels in the liver can often be measured in plasma or serum as a result of normal hepatocyte turnover. Enzyme activity of glutathione *S*-transferase (GST), a biotransformation enzyme

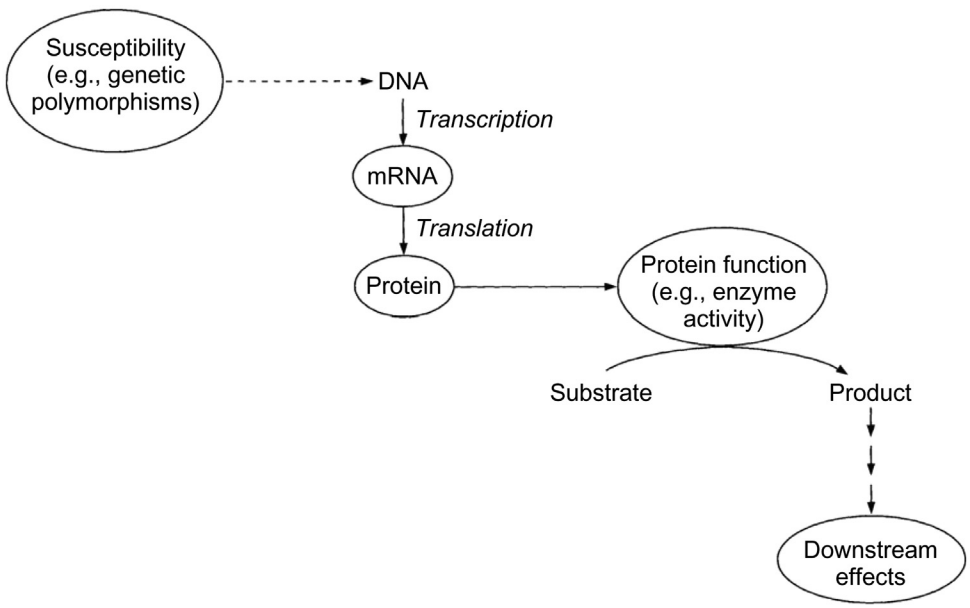

FIGURE 11.2 Direct functional markers of dietary exposure.

important in carcinogen detoxification, can be measured spectrophotometrically in serum [58] or concentrations of the enzyme can be determined in serum by immunoassay [59]. Serum concentration of the GST isoenzyme, GST-α, has been shown to increase when cruciferous vegetables are added to the diet [60]. A limitation of using serum measures of a hepatic enzyme is that the assumption is made that the liver function is normal. Thus, including other measures of liver function in the data collection is important to verify that no underlying hepatic disease is resulting in spurious GST values. In addition, some enzymes are present in isoforms in various tissues. GST-μ, another GST isoenzyme, is present in lymphocytes as well as in liver; therefore, for this isoenzyme, GST activity or protein concentration can be measured in cells extracted from blood samples [59].

Another approach to monitor enzyme activity in vivo is to use a drug probe. Many of the same xenobiotic metabolizing enzymes that metabolize carcinogens also metabolize, and are modulated, by commonly used drugs. The metabolites of these drugs can be monitored in serum, plasma, or urine and used to determine enzyme activities. For example, measuring caffeine metabolites in urine samples collected 4 hours after consumption of a defined caffeine dose allows determination of cytochrome P4501A2, *N*-acetyltransferase, and xanthine oxidase activities [60], and urinary concentrations of the glucuronide and sulfate conjugates of acetaminophen (paracetamol) are used to measure UDP-glucuronosyltransferase and sulfotransferase activities [61]. Drugs can be administered as probes during a nutrition intervention to determine the degree of change in enzyme activity in response to diet and to examine gene−diet interactions [62−64].

Measurement of arachidonic acid metabolism, which involves measuring the concentration of prostaglandins or leukotrienes (metabolic products) or enzymes in the eicosanoid metabolic pathway (i.e., cyclooxygenase), provides another example. Altered arachidonic acid metabolism is among the biochemical activities of nonsteroidal antiinflammatory agents and may also be influenced by antioxidant micronutrients such as vitamin E [65], and quantitative changes in these products or enzymes in tissues serve as biomarkers of this activity [66]. A reasonable amount of biological evidence suggests some role for this enzymatic pathway in colon carcinogenesis [67], but the overall relationship with the disease process is still under investigation.

Similarly, endogenous compounds can serve as probes to monitor enzyme activity. Serum concentrations of the amino acid homocysteine, compared to serum and red blood cell folate concentration, are a more sensitive systemic measure of cellular folate depletion [68,69]. Serum concentrations of homocysteine increase with folate inadequacy because the remethylation of homocysteine requires *N*-5-methyltetrahydrofolate as a cosubstrate [70], which therefore provides a functional marker for folate status. Nonetheless, because homocysteine is at the intersection of two metabolic pathways, remethylation and transsulfuration, deficiencies in other nutrients in these pathways—namely vitamin B_{12}, vitamin B_6, and possibly riboflavin—can contribute to elevated serum homocysteine concentrations [68].

B Biomarkers of Oxidative Stress

Oxidative stress has been suggested to play a role in the pathophysiological disease process in cancer, atherosclerotic cardiovascular disease, and many other acute and chronic conditions [71], although the specific relationship with the disease process remains to be established in most instances. Cellular damage caused by reactive oxygen species, which are generated from cellular respiration, cooxidation during metabolism, and the activity of phagocytic cells of the immune system, is controlled by antioxidant defense mechanisms that involve several micronutrients. Oxidative stress describes the condition of oxidative damage resulting when the balance between free radical generation and antioxidant defenses is unfavorable. Direct measurement of active oxygen and related species in biological samples is challenging, mainly because these compounds have short half-lives. Thus, the oxidative stress biomarkers used in human studies are typically adducts or end products that reflect reactions that have occurred between free radicals and compounds such as lipids, proteins, carbohydrates, DNA, and other molecules that are potential targets [72].

One frequently described assay used as an oxidative stress biomarker is the thiobarbituric acid reactive substances (TBARS) assay. The TBARS assay basically quantifies a product of malondialdehyde, which presumably reflects lipid hydroperoxides in the sample. Direct measurement of malondialdehyde in biological samples using HPLC has also been examined as an alternative approach, and plasma malondialdehyde concentrations have been reported to respond to alterations in antioxidant nutrient status, albeit not consistently [72].

Measurement of breath pentane is another biomarker of oxidative stress that has been utilized in human studies [73]. The approach basically involves collecting exhaled air for the measurement of the products of peroxidation of unsaturated fatty acids, a portion of which is volatile and released in the breath, using gas chromatography methods. However, the specific measurement methodologies vary a great deal and are not always reliable, and standardization of the procedure and knowledge of various influencing factors are needed to improve the usefulness of this approach [72].

Another biomarker of oxidative stress is urinary 8-hydroxydeoxyguanosine (8OHdG), which can be measured by several different methods with high sensitivity [74]. The 8-hydroxylation of the guanine base is a frequent type of oxidative DNA damage, and 8OHdG is subsequently excreted without further metabolism in the urine after repair in vivo by exonucleases. In previous studies, certain demographic factors and physiological characteristics such as gender and body mass [75] have been observed to influence urinary 8OHdG concentration, so these factors may need to be considered in interpretation. Urinary 8OHdG is increased in association with conditions known to be characterized by increased oxidative stress, such as smoking, whole-body irradiation, and cytotoxic chemotherapy [74–76]. Urinary 8OHdG (unadjusted or adjusted for urinary creatinine) has also been observed to decline in response to a high vegetable and fruit diet intervention in human subjects [77], which is particularly interesting because this type of diet has been suggested to promote reduced oxidative stress [13,77,78].

Prostaglandin-like compounds produced by nonenzymatic free radical-catalyzed peroxidation of arachidonic acid, termed F_2 isoprostanes, are currently of great interest as useful biomarkers of oxidative damage [79]. Specific gas chromatography–mass spectrometry (GC-MS) assays for the measurement of some of these compounds, such as iPF2a-III (also called 8-iso-$PGPF_2$) and iPF_2-VI, have been developed and used to quantify the compounds in urine and blood samples. These markers have been shown to be less variable than 8OHdG [80]. Elevated levels have been observed in plasma and urine samples from subjects under a wide variety of conditions of enhanced oxidative stress [81], and the measure can be altered in dietary intervention studies designed to reduce oxidative damage [82].

Another approach to measuring DNA oxidative damage that has been evaluated for use in human nutrition intervention research is the measurement of 5-hydroxymethyluracil levels in DNA in blood. 5-Hydroxymethyluracil is produced when DNA is exposed to oxidants, is relatively stable compared to other oxidation products, and can be quantified with GC-MS [83,84]. At the same time, its high intraindividual variation reduces its utility as a biomarker [85].

Oxidative damage to low-density lipoprotein (LDL) has been specifically linked to atherogenesis, and in an application of this biological activity, measurement of LDL oxidation ex vivo has been used in clinical studies as a biomarker of oxidative stress [86]. Basically, this process involves isolating the LDL fraction from a blood sample, exposing this fraction to oxidants, such as Cu^{2+}, and measuring the time lag before oxidation. Also, various specific methodologies are used across laboratories, and the lack of standardization in the approaches in use constrains the ability to make comparisons across studies.

Several other approaches to measuring biomarkers of oxidative stress have been proposed and are under study, and the reader is referred to a review of this topic [72]. Because of their inherent variability and uncertain responsiveness to dietary manipulations, clinical researchers often employ a panel of potential measures of oxidative stress rather than relying on a single indicator [77].

C Biomarkers of Immune Function

The human immune system is a complex and highly interactive network of cells and their products have a central role in protecting against various external disease-promoting factors and perhaps against malignant cells. Many of the components of the immune system can serve as biomarkers and are monitored in vivo or ex vivo [87,88]. Because of the complexity of the system, the selection of assays should be closely aligned with the research question being asked. Furthermore, multiple parameters need to be measured; one single biomarker is inadequate to monitor immune function.

Cell-mediated immune variables include the absolute amounts or ratios of various white blood cell (WBC) types (e.g., total counts, WBC differentials, T cell subsets) and measures of T cell function (e.g., lymphocyte proliferation, cytokine release from mitogen-stimulated cultures, cytotoxic capacities, and delayed-type hypersensitivity (DTH)). Both number and activity of natural killer cells (one of the cell types that play an important role in immune surveillance) are used as biomarkers in nutrition intervention trials [89,90]. Cytokines (e.g., the interleukins) are soluble factors released by immune cells, which control and direct the function of other immune effectors. Some of these have been used as markers of immune response in randomized trials of vitamin supplementation [90,91].

An in vivo functional test, the DTH skin test, is widely used to monitor the immune system in humans, including in studies of dietary modulation of immune function [92]. The test measures the capacity of an individual's immune system to mount a response to antigenic stimulation. The DTH test typically involves the simultaneous intradermal application of one or several DTH antigens. These antigens elicit an immunological reaction involving the release of lymphokines by antigen-sensitized T cells. These compounds, in turn, activate macrophages, which release inflammatory mediators, resulting in measurable skin induration.

D Biomarkers of Bone Health

Bone mass measurements and biomarkers of bone turnover are used as functional indices of bone health and, to a certain extent, can also be used as markers of the

adequacy of calcium intake. Measures of bone mass include bone mineral content (i.e., the amount of mineral at a particular skeletal site, such as the femoral neck, lumbar spine, or total body) and bone mineral density (BMD; i.e., bone mineral content divided by the area of the scanned region). Both are strong predictors of fracture risk [93–95]. Controlled calcium intervention trials that measure change in BMD provide evidence for the intake requirement of calcium [96] (see Chapter 44, Osteoporosis: The Early Years, and Chapter 45, Osteoporosis in Adults, for more details).

Biochemical markers of bone turnover predict bone mass changes and fracture risk and respond to dietary calcium intake [96]; thus, they provide some promise for a biochemical indicator of calcium status. Unlike BMD, they reflect more subtle changes in bone metabolism. Bone turnover is the cyclical process by which the skeleton undergoes renewal by a coupled, but time-separated, sequence of bone resorption and bone formation [97]. Markers of bone turnover rely on the measurement in serum or urine of enzymes or matrix proteins synthesized by osteoblasts or osteoclasts that spill over into body fluids, or of osteoclast-generated degradation products of the bone matrix [96]. Currently, serum levels of skeletal alkaline phosphatase and osteocalcin are used as markers of bone formation, and products of collagen degradation measured in urine are used to measure bone resorption. These markers exhibit substantial short-term and long-term fluctuations related to time of day, phase of menstrual cycle, and season of the year, as well as other factors that alter bone remodeling (e.g., exercise) [98].

E Biomarkers of Cell Turnover

Cellular markers of proliferation, differentiation, and apoptosis (i.e., programmed cell death) can be useful as biomarkers in research focused on nutritional factors and cancer, although the measured effect is a general indicator of an altered cell growth regulation effect. Use of such markers is severely restricted by the difficulty accessing the tissue of interest. Consequently, research in this area has been limited primarily to tissue available via endoscopic or fine-needle biopsy procedures (e.g., gastrointestinal tract, breast, and prostate).

As a general rule, increased proliferation of undifferentiated cells defines one aspect or characteristic of carcinogenesis, and in colon cancer, this relationship has been well-established. For example, cell proliferation occurs at the base of the colonic crypts, and as cells migrate from the crypts to the luminal surface, they become increasingly differentiated and mature and lose their proliferative capabilities [99]. The shift in which the proliferative zone extends to the surface so that cells on the luminal surface retain proliferative capabilities and are immature and underdifferentiated may be considered a field defect that sets the stage for current and future neoplastic changes [99–101]. Early work in this area relied on the incorporation of tritiated thymidine or bromodeoxyuridine into the DNA of dividing cells during incubation of a biopsy specimen. These methods required that the tissue be freshly obtained, so the cells were viable and replicating. Often, label incorporation was incomplete. Now, with increased sensitivity in immunohistochemical techniques, proteins present in proliferating cells (e.g., proliferating cell nuclear antigen (PCNA) and Ki67) are used more widely to quantify proliferative activity in tissue specimens. Labeling indices involving tritiated thymidine and PCNA have been used to quantify the proliferative activity in colonic mucosal samples from human subjects [102] and have been used successfully as end points in several nutrition intervention studies to prevent colon cancer [103]. These indices are being further refined by staining for proteins present during apoptosis (e.g., Bax and Bcl-2) and in differentiated cells in order to provide a more complete picture of cell dynamics.

Adoption of aberrant crypt foci (early morphological changes in colonic epithelium that are considered potential precursors of adenomatous polyps) as biomarkers in humans is an example of how improvements in technology have led to the adoption of a biomarker that until recently could only be used in animal studies. Development of magnifying endoscopes with improved resolution now allows investigators to monitor aberrant crypt foci in colon tissue samples from healthy humans [104].

IV BIOMARKERS OF GENETIC SUSCEPTIBILITY

The health of individuals and the population in general is the result of interaction between genetic and environmental factors. For the great majority of human diseases, purely genetic or purely environmental etiologies are insufficient to explain individual variability in occurrence, prognosis, or outcome [105]. This is especially the case with chronic diseases, such as heart disease and cancer. Genetically determined susceptibility factors alter disease frequency or treatment response through variations in the DNA coding sequences of genes. As a result, genetically susceptible individuals produce proteins that are structurally different from those of individuals who are not at increased risk of disease, or they produce them in greater or lesser amounts.

By genetic standards, traits with a frequency of between 1/100 and 1/10,000 in a population are considered uncommon, and rare traits are those with a frequency of less than 1/10,000 [106]. Typically, these are low-prevalence and high-penetrance genes (e.g., genes associated with familial

cancers). Common genetic traits are those in which the least common allele is present in at least 1% of the population [106]. Traits with this characteristic are known as genetic polymorphisms. They include the high-prevalence, low-penetrance genes—"susceptibility genes"—thought to contribute to disease risk.

In cases in which specific genetic mutations or variations may indicate disease risk or progression or may be modified by nutritional factors, genetic markers can also be useful biomarkers. Various molecular techniques have been developed to help characterize genetic abnormalities or differences. Genetic factors are important to consider in nutrition research for several reasons. One reason is that it is increasingly evident that genetic polymorphisms may contribute substantially to differences in the response to environmental and dietary exposures [107]. For example, genetic variations in the expression of the xenobiotic metabolizing enzymes may mediate the potentially mutagenic effect of heterocyclic amines (obtained from meat cooked at high temperature) [108] (see also Chapter 34: Nutrition and Cancers of the Breast, Endometrium, and Ovary). Also, results from laboratory animal studies suggest that dietary modifications can promote alterations in genetic factors [109] so that measuring genetic abnormalities may be considered as an approach to demonstrating a biological link between dietary factors and disease risk.

Some polymorphic traits may only be important in the presence of a particular dietary exposure. For example, carrying the 5,10-methylenetetrahydrofolate reductase thermal-labile variant has been shown to be a risk factor for colorectal adenomatous polyps, but only in the context of low-folate, vitamin B_{12}, and vitamin B_6 intake [110].

Given that a goal of nutrition research and interventions is the prevention and treatment of disease, genetic markers may aid in this effort by identifying population subgroups at high risk of disease in the presence of particular dietary exposures. Genetic susceptibility markers may also strengthen our understanding of disease by focusing attention on possible pathways of disease causation and progression. There is considerable heterogeneity in disease risk within populations; thus, markers of susceptibility may also help to clarify associations between dietary exposures and diseases within population subgroups [111].

V METABOLOMICS FOR BIOMARKER DISCOVERY

Metabolomics involves the quantitative and qualitative study of a large number (e.g., 400−800) of small molecules (typically less than 850 Da) in biological fluids, including serum or plasma and urine. Metabolomic profiles can be measured by using GC-MS, LC-MS, or nuclear magnetic resonance spectroscopy. This metabolic phenotyping integrates exposure and functional biomarkers, and it allows the investigation of biomarkers of exposures as well as disease processes. The advantage of nutritional studies is the ability to investigate an array of dietary variables rather than single exposures; furthermore, an individual's metabolic profile reflects environmental and genetic factors as well as gut microbiota, all of which play an important role in diet and metabolism.

In controlled feeding studies, metabolomics has identified metabolic signatures for consumption of diets varying in composition of fats [112], proteins [113], and phytochemicals [114,115], as well as black versus green tea [116]. According to van Duynhoven et al. 2010, intestinal microbiota in humans are responsible for biotransformation of polyphenol-rich foods into more bioavailable forms for the body to have an effect [117]. Additional focused and comprehensive efforts are underway to identify metabolites related to diet, physical activity, energy balance, and disease end points [118−122]. For example, recent studies have shown that the increased presence of branch-chained amino acids (BCAA) induces insulin resistance, possibly having to do with the signaling pathway and metabolism of BCAAs [119]. Although preliminary data suggest that only 20% of metabolites are affected when samples are nonfasting [123,124], more studies are required to investigate the sources of variation in metabolic profiles between biological fluids, as well as inter- and intraindividual variation.

VI CRITERIA FOR SELECTING AND USING BIOMARKERS

When a candidate biomarker is identified, certain basic considerations need to be addressed before it can be adopted for use in research or in a clinical setting [125]. These considerations relate to the reliability of the laboratory assay, the biological relevance of the marker, and the characteristics of the marker within a population. Whether or not a particular established marker is used also depends on the purpose the marker will serve.

Development of a biomarker usually builds on scientific knowledge from various types of laboratory studies, including tissue culture and animal studies. In the laboratory, an initial priority is to determine a marker's reliability or reproducibility. Assay performance can be evaluated using coefficients of variation (CV%; [SD/mean] × 100) to estimate within- and between-batch precision; these are measures of analytical, laboratory performance and do not reflect intra- or interindividual variation. The within-batch precision is determined by

dividing single samples into multiple aliquots and analyzing them together. Between-batch precision is determined by analyzing multiple aliquots on separate days. The acceptable numerical values for the laboratory CV% are difficult as the degree of error depends on the use of the biomarker data. For epidemiological studies, if the goal is to establish a stable estimate of the group mean, an acceptable CV% may depend on the number of samples available, the mean concentration of the biomarker, and between-individual variation [126]. Techniques of quality control [127,128] and statistical methodologies for managing quality control data [129] have been established and are used in clinical laboratories.

Biomarkers should be relatively easy to measure and require relatively noninvasive techniques of tissue sampling. This requires that the biological relevance or validity—i.e., the relationship between biomarkers in tissues readily available for human monitoring (e.g., peripheral blood) to those in target tissue (e.g., lung and liver) and the relationship between the biomarker and the disease or exposure being studied—of the measure be established. One example is the use of serum ferritin as a marker of body iron stores. Serum ferritin was validated as a measure of iron stores against bone marrow examination for stainable iron, the criterion—but very invasive—method for measuring iron stores [130]. As a result, serum ferritin has been adopted as a simple, quantitative biomarker of iron stores in otherwise healthy individuals.

If a particular biomarker is to be used as a measure of dietary exposure, it must be evaluated with respect to its sensitivity to that intake. Several approaches, both observational and interventional, can be used to define the relationship between long-term dietary intake and biological levels. Investigators can rely on geographical differences in exposure: Tissue samples from areas of known nutrient deficiency of a specific nutrient can be compared with samples from average and high-exposure areas. This approach has the advantage that it can reflect the long-term intake of a settled group of individuals; however, identifying and controlling confounding factors is a major challenge [126]. Another observational approach is to establish within individuals the relation between a dietary exposure and the biochemical marker. Participants for such a study can be selected randomly or can be selected specifically to maximize the range of intakes. Rigorous testing of the relationship between intake of the dietary factor and a biomarker under controlled dietary conditions is also valuable to establish dose−response relations; however, these trials are usually limited to weeks or months, and if they involve extensive changes to usual diet, blinding of participants may not be feasible.

Depending on the biomarker, significant variability can be seen in a biomarker. Sources of variation can be internal (e.g., age, sex, genetics, body build, and biological rhythm) or environmental (e.g., diet, season, time of day, immobilization, exercise, and drugs). These can contribute to both within- and interindividual variation. Additional external sources of variation, beyond laboratory accuracy and precision, can include an individual's posture during sample collection and sample handling and storage; protocols should be established to minimize these latter sources of variability.

Selection of a biomarker is dependent in part on its use. A biological indicator that is going to be used as a measure of a dietary exposure in an epidemiological study needs to be a valid representation of long-term intake [126]. Repeated sampling and measurement of a biomarker over time can provide some estimate of the within-individual variability and, therefore, the likelihood that the biomarker is a stable estimate of long-term intake. If repeated measures of a biochemical indicator vary substantially over time in the same individuals, then a single measure will not accurately reflect true, long-term intake [126]. This lack of consistency may occur because diet has changed over the sampling interval or because the measure is overly sensitive to short-term influences, such as recent intake. When using dietary constituents or their metabolites as biomarkers, an understanding of the metabolism and pharmacokinetics of the compound and the frequency of exposure will help to establish the utility of the measure as a biomarker of long-term intake.

A nutrition intervention study may require a biomarker that is a short-term measure of response to treatment. A biomarker that is to serve as a short-term measure of response needs to change within the time frame of the intervention. For example, serum folate provides a measure of recent folic acid exposure; however, erythrocyte concentrations are dependent on the life span of the cells and therefore will not reflect short-term changes in dietary folate. Serum folate concentrations decline within 3 weeks after the initiation of a low-folate diet, whereas erythrocyte folate concentration remains in the normal range for at least 17 weeks [131].

Additional practical considerations in the use of established biomarkers include the ability to conveniently access the body compartment for measurement, the procedures necessary to collect and process the sample, the burden to study participants or patients, and the resources for laboratory analysis. For example, multiday collections of feces or urine can be a major burden for many individuals. In addition, they can result in incomplete collections, which also compromise the final results. An accurate quantification of vitamin C or folate in a circulating body pool requires processing steps that must be conducted immediately after blood collection to preserve the sample appropriately and prevent degradation that would otherwise make the resulting measurement inaccurate. These extra steps can add time and effort to the labor

of blood processing. Furthermore, the complexity of an assay method can vary from the ability to analyze hundreds of samples a day at a cost of a few dollars per sample to a labor-intensive, week-long process that costs hundreds of dollars per sample.

The ability to measure particular biomarkers is also often linked to technological challenges and existing capabilities. For example, HPLC (developed in the 1970s) and improved separation and detection technologies that are currently emerging facilitate the quantification of many micronutrients and other dietary constituents that are present in very low concentrations in biological samples. Similarly, immunoassays allow for quantitation of phytochemicals, proteins, and so on in small volumes of serum or plasma, whereas previous methods required substantial quantities of sample. The development of microarray technology has provided the ability to analyze the expression profiles for thousands of genes in parallel [132,133]. This technique will rapidly advance knowledge regarding the mechanisms by which nutrition and diet affect disease risk; however, its application in intact humans will still be limited by access to the tissue of interest and the capacity to detect small but relevant changes in gene expression in response to diet.

A summary of the ideal characteristics of a biomarker, the assay involved, as well as study-specific questions is given in Table 11.3. Many of the prerequisites of a potential biomarker depend on the research question and study design.

TABLE 11.3 Criteria for Selecting Biomarkers

Qualities of a good biomarker
Sensitive
Specific
Biologic relevance to target tissue
Accessible in biospecimen—not invasive
Easy to measure
Applicable to many populations
Qualities of a good biomarker assay
Reliable
Reproducible
Sensitive
Identify sources of variability in the biomarker
Interindividual variation (e.g., age, sex, genetics, diet, lifestyle, season, time of day, drugs)
Intraindividual variation
The effect of sample handling and storage; e.g., is the biomarker stable during long-term storage?
Identify the type of biomarker needed
Is the biomarker applicable to the setting? For example, cohort studies usually have biospecimens from only one time point—Is a single measure representative and sufficient?
Is the assay applicable to the setting? For example, the assay would need to have relatively high throughput for cohort studies, whereas for small intervention studies this would be less important.
Does the study require a short-term or a long-term biomarker?
Does the biomarker reflect a dose–response relation?
Do the biospecimens need to be collected after fasting or should the individuals be nonfasting?

VII SUMMARY

The use of biomarkers in humans is an integral component of nutrition intervention research. Biochemical measurements of dietary constituents in blood or other tissues can provide a useful assessment of the intake of certain dietary factors. However, for some nutrients, functional markers, or direct functional indices, provide a better estimate of the significance of the true status for a nutrient. More general functional indices and indicators of biological activity relating to processes associated with disease risk are important for establishing the relationships between diet and disease prevention and response to treatment.

The development of biomarkers continues at a rapid pace. New types of markers are being proposed constantly, and analytical techniques for existing markers are improved. This advancement requires establishing the accuracy, reliability, and interpretability of the biomarkers; obtaining data on marker distributions within different age and sex groupings in normal populations; determining the extent of intraindividual variation in markers with respect to tissue localization and persistence; and assessing the contribution of genetic and acquired susceptibility factors to interindividual variability.

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Chapter 12

Nutrition Guidelines to Promote and Maintain Health

Cheryl A.M. Anderson[1] and Barbara Millen[2,3]

[1]*University of California at San Diego, La Jolla, CA, United States,* [2]*Boston University School of Medicine, Boston, MA, United States,* [3]*Millennium Prevention, Inc, Westwood, MA, United States*

I INTRODUCTION AND OBJECTIVES

To promote and maintain health in the US population, and in many other parts of the world, numerous authoritative medical and public agencies have developed national health policies and expert clinical practice guidelines. All of these acknowledge that improvements in lifestyle behaviors such as healthful dietary patterns, physical activity, smoking abstinence, and alcohol moderation (if consumed, and in adults only) are the cornerstones of chronic disease prevention and health promotion.

A Importance of Nutrition Guidelines That Support Chronic Disease Reduction and Health Promotion

National nutrition guidelines are supported by a large and robust body of epidemiological and clinical research that strongly supports the importance of optimal nutrition and other healthy lifestyle behaviors in reducing risks for major chronic diseases and promoting other favorable health outcomes. This evidence base not only confirms that diet is related to the prevention and treatment of many chronic diseases, but also demonstrates the efficacy and impact of preventive nutrition and lifestyle behavioral interventions on a wide range of other health outcomes. Practicing healthy lifestyle behaviors is central to reducing chronic disease risk and improving overall health and well-being but, behavior modification is difficult. Interventions must be targeted, intensive, and multidisciplinary in nature to increase their effectiveness at addressing the complex levels of influence on diet and other lifestyle behaviors. Thus, guidelines and policies should play an important role in facilitating adherence to healthful behaviors.

In the United States, federal nutrition and health policies are designed to influence and shape nutrition, healthcare and public health systems throughout the nation as well as other sectors, such as food and agriculture. For example, the *Dietary Guidelines for Americans* (DGAs) are public policy directives that affect the nature and delivery of programs and services of the US Department of Agriculture (USDA) as well as US Department of Health and Human Services. The DGAs and other national nutrition policies and expert clinical practice guidelines affect many programs and services such as the large federal framework of food and nutrition assistance programs as well as the US healthcare and public health systems. In addition, they influence research priorities in the National Institutes of Health (NIH) and research and educational initiatives of USDA, and the Centers for Disease Control and Prevention (CDC). They can also influence regulations developed within the Food and Drug Administration.

B Public Health Approaches Can Create Environments Where Healthful Diets and Lifestyles Are Normative

Nutrition and other lifestyle behaviors are determined by multiple levels of influence. The 2015–2020 DGAs utilized the socio-ecological model [1–2] to identify the wide-ranging set of factors that affect diet and other lifestyle behaviors at individual, socio-cultural, environmental, multisectoral, and policy levels. The DGAs note that public health approaches that combine interventions aimed at

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individual and populations will promote improvements in lifestyle behaviors. Multicomponent interventions can be guided by the current, sound research evidence base and "best practice" models, particularly those utilizing a socio-ecological approach. Most interventions will be comprehensive in nature, guided by multidisciplinary teams, and more recently, will incorporate cross-sectoral collaborations and public–private partnerships. The 2015–2020 DGAs also emphasized that interventions are most effective when they are designed to target the needs and preferences of the individuals and communities served. Of note, successful public health efforts such as those addressing automobile safety and tobacco use have used broad-based, inclusive approaches consistent with a socio-ecological model [1].

C Demographic Differences in Health Outcomes and Trajectories Support Tailored Nutrition Interventions

Increasingly, experts advocate that lifestyle interventions should be personalized to the biological and other personal needs and preferences of individuals and facilitate changes in the environments and settings in which populations work, are educated and trained, and seek recreation. Expert recommendations on personalized approaches to preventive behavioral intervention are guided by research showing that lifestyle behavior profiles and metabolic and disease-related risks vary within as well as across populations and are inextricably linked. For example, the Framingham Nutrition Studies found that adult males and females have differing patterns of lifetime weight gain and varying rates of development of overweight, obesity, and abdominal obesity in early and late adulthood [3–5]. The Framingham Nutrition Studies also found that 10 distinct, habitual dietary patterns with differing food composition and nutrient quality could be identified and were shown to be associated with metabolic risk profiles and risks for development of: overweight and obesity, abdominal obesity, metabolic syndrome, heart disease, hypertension, and stroke [3–5]. Framingham investigators interpreted these findings, combined with results from other epidemiological studies and the emerging and extensive research from randomized clinical trials (RCTs) in diverse populations, as underscoring the importance of gender-specific, individualized prevention and treatment strategies.

Consistent with this research and a large body of current, high-quality research, the 2015–2020 US DGAs and the recent American Heart Association/American College of Cardiology/The Obesity Society (ACC/ACC/TOS) Guidelines for the Management of Overweight and Obesity [6] and the ACC/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk [7] recommend that preventive nutrition and lifestyle interventions be utilized in clinical and public health systems and other community settings. They also advocated that they be targeted to the biological needs and personal preferences of individuals and populations served.

D Chapter Objectives

This chapter is focused on nutrition guidelines to maintain and promote health. The aims are to review:

- guidelines on human nutrient requirements;
- federal nutrition-related health policies and related expert guidelines on health promotion and chronic disease prevention; and
- how nutrition policies and expert recommendations can inform professional practices to promote and maintain health.

II GUIDELINES ON HUMAN NUTRIENT REQUIREMENTS

A Dietary Reference Intakes

The Dietary Reference Intakes (DRIs) offer guidance on the level of nutrient intake that will promote health. They are comprised of four categories of values that help promote health, prevent disease, and provide guidance on consumption levels. The four categories are: the Estimated Average Requirement (EAR), the Recommended Dietary Allowance (RDA), the Adequate Intake (AI), and the Tolerable Upper Intake Level (UL).

1 Process for Establishing DRIs as Guidelines on Human Nutrient Requirements

The DRIs play a critical role in helping Americans meet their nutritional needs. To date, they have been developed by the Food and Nutrition Board of the Institute of Medicine, in conjunction with substantial input from nutrition professionals [8]. In the 1990s, the DRIs expanded and replaced the RDAs, which had been used since 1941, because of a broader evolution that was taking place in the field of nutrition science [9]. The dietary reference values were published in a series of reports titled *Dietary Reference Intakes* and were released between 1997 and 2005. The DRIs are updated, as needed, on an ongoing basis by experts at the Food and Nutrition Board of the National Academies of Medicine.

2 DRIs Serve as a Major Component of Federal Nutrition Policy

As noted above, the DRI is an umbrella term for four types of nutrient recommendations: EAR, RDA, AI, and

UL. The EAR is the intake that would be adequate to meet the nutritional requirements/needs of approximately 50% of a healthy population; the RDA is calculated from the EAR by adding two standard deviations of the requirement distribution. The RDA is thus the level of intake that would be adequate for 97.5% of the population. The AI is used when the scientific data are not sufficient to set an EAR and its associated RDA. The UL represents the upper level of intake that poses a low risk of adverse effects, and usual intakes above the UL are not recommended.

DRIs have been set for energy, 14 vitamins, 15 minerals, and 7 macronutrients. A summary of all DRI values, except EAR, is provided in the Appendix. Two reports have been released to address appropriate ways to use the DRIs—one assesses nutrient intakes [10] and the other on planning nutrient intakes at individual and programmatic levels [11]. The RDA (or the AI if an RDA is not available) is the appropriate target for guidance to consumers on healthy nutrient intakes, and the goal is to reduce to a very low level the likelihood that an individual's intake is inadequate. Thus achieving the RDA or the AI means that the risk of inadequate intake is very low. A UL should never be considered a target intake and health professionals may guide consumers in using the UL to ensure that nutrient intakes are not too high.

3 Importance of DRIs for Setting Nutrition Guidelines for Prevention and Treatment of Chronic Disease

The four primary uses of the DRIs are to assess the intakes of individuals, assess the intakes of population groups, plan diets for individuals, and plan diets for groups. They are used in planning for food assistance programs, food labeling, fortification efforts, food safety assurance, institutional food planning, military food and nutrition planning, and in developing new products or modifying existing food products.

The DRIs play a critical role in promoting and maintaining health through their link to the *DGAs* (the DGAs are discussed in greater detail below) [12]. The guidelines and the materials that support them are designed to help Americans meet the DRIs, primarily through consuming foods rather than supplements.

III FEDERAL NUTRITION-RELATED HEALTH POLICIES

A The Dietary Guidelines for Americans

Every five years, the US Departments of Health and Human Services (HHS) and Agriculture (USDA) are mandated under the 1990 National Nutrition Monitoring and Related Research Act (Public Law 101-445, 7 U.S.C. 5341 et seq) to jointly publish the DGAs [13]. The DGAs are federal policy guidelines that must be based on the preponderance of current scientific and medical knowledge and contains nutritional and dietary recommendations for the general public. The DGAs have far-reaching impact.

B Uses and Impact of the DGAs

The DGAs are used in developing federal food and nutrition programs, educational initiatives, research and programmatic priorities, and health policies and programs within the agencies that fall within the very broad jurisdictions of HHS and USDA. Among the many realms affected by the DGAs are the CDC, NIH, the US Public Health Service, the US healthcare and public health systems, the Food and Drug Administration (which regulates food labeling), the Agricultural Research Service, the Supplemental Nutrition Assistance Program (SNAP) and Nutrition Program for Women, Infants and Children (WIC), School Lunch and Breakfast Programs, Older Americans Act Nutrition Program, etc. The US military may also use the DGA to guide food and nutrition services as well as health-related programs.

The DGAs are prepared for a professional audience and are intended to guide services and interventions delivered to individuals aged 2 years and older and their families and promote consumption of a healthy, nutritionally adequate diet. Given the link between diet and physical activity and population health, the DGAs endorse healthy dietary patterns and physical activity. Additional audiences who may use *Dietary Guidelines* information to develop programs, policies, and communication for the general public include businesses (i.e., corporate wellness initiatives), schools (e.g., foodservice, curriculum, and other services), community groups (environmental and population initiatives, etc.), media, the food industry, and state and local governments.

C Overview of Process Used to Develop 2015 DGAs

The development of the 2015−2020 DGAs began with the appointment of a Dietary Guidelines Advisory Committee (DGAC) composed of nationally recognized experts in the field of nutrition and health. An external advisory committee is not mandated by law but has been appointed since the 1980s. The DGAC was charged to provide independent, science-based advice and recommendations for development of the guidelines. In so doing, the committee was asked to assess the *DGAs*, 2010, and determine where the new scientific evidence was available to inform revisions to current guidance or

suggest new guidance, in particular food-based recommendations of public health importance for Americans aged 2 years and older. The committee process was to be completed in 2 years and culminate in the submission of a report on scientific recommendations to the Secretaries of HHS and USDA. DGAC responsibilities did not include translation of the recommendations into 2015 DGAs; however, USDA and HHS are mandated to translate the DGAC recommendations into public policy in the DGAs.

The 2015 DGAC was a 14-member committee of scientists nominated through a public process and appointed by the Secretaries of HHS and USDA. DGAC members are recognized as experts in a broad range of domains, including food and nutritional sciences, medicine, epidemiology, nutrition and health policy, public health, and related areas. All DGAC members served without compensation and were fully vetted according to strict federal guidelines with regard to potential conflicts of interest pertaining to their committee responsibilities.

D Scientific Approach of the DGAC

The 2015 DGAC initially identified five major research themes: (1) food and nutrient intakes and health—current status and trends; (2) dietary patterns, foods and nutrients, and health outcomes; (3) diet and physical activity behavior change; (4) food and physical activity environments; and (5) food sustainability and food safety. Five subcommittees were then established to formulate research question priorities. In addition, three cross-cutting working groups were formed to address issues that transcended the subcommittee themes: (1) sodium, (2) saturated fatty acid (SFA), and (3) added sugars. Each 2015 DGAC member served on at least two of the subcommittees and one of the cross-cutting working groups. An oversight Science Review Subcommittee composed of the DGAC Chair, DGAC vice-chair, and two DGAC members who participated on the 2010 DGAC as well as subcommittee and working group chairs were formed to provide oversight to the process.

The procedures used to prepare the 2015 DGAC Scientific Report were systematic, transparent, and thorough and provided multiple opportunities for public input through oral testimony and website submissions. Rigorous and strict rules for DGAC meetings and communications were established and were in keeping with requirements of the Federal Advisory Committee Act [14]. All meetings of the DGAC were announced in advance in the Federal Register. At least one federal staff member representing the Designated Federal Officer was part of all subcommittee and working group meetings, conference calls, and emails. All meetings of the 2015 DGAC were made available via real-time broadcasts and, along with public comments, are archived online [15]. In February 2015, the Scientific Report of the 2015 DGAC was submitted to the Secretaries of HHS and USDA and the committee completed its assignment. The Scientific Report of the 2015 DGAC can be found online [13]. Upon completion of its report, the DGAC was disbanded as mandated by Congress. In February 2016, the 2015–2020 DGAs were published by HHS and USDA, thereby completing the mandated process of translating the DGAC Scientific Report into public policies.

E Summary of the 2015–2020 DGAs

In large part, the DGAs are consistent with the DGAC report and recommendations which underscored the importance of addressing the highly prevalent diet- and lifestyle-related diseases in the US population (including overweight and obesity, cardiovascular disease (CVD), diabetes, and diet-related cancers); improving the quality and composition of the American dietary pattern; making gradual changes in dietary patterns to be healthier and increasing physical activity; utilizing sound and effective methods of individual and population level behavior change to promote healthy dietary patterns and physical activity; and maintaining food quality and safety with best practices. There are two major areas in which the DGAC and DGAs differ. First, the 2015 DGAC recommended not to bring forward the 2010 DGAs limit on dietary cholesterol but did model healthy dietary patterns with relatively low cholesterol intake. Additionally, the Secretaries of USDA and HHS announced that the DGAC recommendations on the important topic of environmental sustainability would not be addressed in the 2015–2020 DGA. The 2015–2020 DGAs are summarized in brief below.

The *2015–2020 Dietary Guidelines* provide five overarching guidelines (Table 12.1) that encourage healthy dietary patterns, recognize that individuals will need to make shifts in their food and beverage choices to achieve a healthy pattern, and acknowledge that all segments of our society have a role to play in supporting healthy lifestyle choices, particularly dietary and physical activity. Key recommendations for healthy eating patterns are shown in Table 12.2. The 2015 DGAs indicate that the recommendations for healthy eating patterns should be applied in their entirety given the interconnected relationship of the various dietary components.

The 2015 DGAs also acknowledge that a healthy eating pattern is not a rigid prescription, but rather, an adaptable framework in which individuals can enjoy foods that meet their personal, cultural, and traditional preferences and fit within their budget. Three basic healthy dietary patterns were modeled (i.e., Healthy US Style, Healthy Vegetarian, and Healthy Mediterranean-style) in the DGAC report and are included in the 2015–2020 DGA

TABLE 12.1 Five Overarching Guidelines from the 2015–2020 US *DGAs*

1. Follow a healthy eating pattern across the lifespan. All food and beverage choices matter. Choose a healthy eating pattern at an appropriate calorie level to help achieve and maintain a healthy body weight, support nutrient adequacy, and reduce the risk of chronic disease.
2. Focus on variety, nutrient density, and amount. To meet nutrient needs within calorie limits, choose a variety of nutrient-dense foods across and within all food groups in recommended amounts.
3. Limit calories from added sugars and SFAs and reduce sodium intake. Consume an eating pattern low in added sugars, SFAs, and sodium. Cut back on foods and beverages higher in these components to amounts that fit within healthy eating patterns.
4. Shift to healthier food and beverage choices. Choose nutrient-dense foods and beverages across and within all food groups in place of less healthy choices. Consider cultural and personal preferences to make these shifts easier to accomplish and maintain.
5. Support healthy eating patterns for all. Everyone has a role in helping to create and support healthy eating patterns in multiple settings nationwide, from home to school to work to communities.

TABLE 12.2 Key Recommendations from the 2015–2020 US Dietary Guidelines[a]

Consume a healthy eating pattern that accounts for all foods and beverages within an appropriate calorie level. A healthy eating pattern includes:

- A variety of vegetables from all of the subgroups—dark green, red and orange, legumes (beans and peas), starchy, and other
- Fruits, especially whole fruits
- Grains, at least half of which are whole grains
- Fat-free or low-fat dairy, including milk, yogurt, cheese, and/or fortified soy beverages
- A variety of protein foods, including seafood, lean meats and poultry, eggs, legumes (beans and peas), and nuts, seeds, and soy products
- Oils

A healthy eating pattern limits:

- SFAs and *trans*-fats, added sugars, and sodium

Consuming the specified limits of these dietary components can help individuals achieve healthy eating patterns within calorie limits:

- Consume less than 10% of calories per day from added sugars.
- Consume less than 10% of calories per day from SFAs.
- Consume less than 2300 mg per day of sodium.
- If alcohol is consumed, it should be consumed in moderation—up to one drink per day for women and up to two drinks per day for men—and only by adults of legal drinking age.

In tandem with the recommendations above, Americans of all ages—children, adolescents, adults, and older adults—should meet the *Physical Activity Guidelines for Americans* to help promote health and reduce the risk of chronic disease. Americans should aim to achieve and maintain a healthy body weight. The relationship between diet and physical activity contributes to calorie balance and managing body weight.

[a]*The* Dietary Guidelines*' key recommendations for healthy eating patterns should be applied in their entirety, given the interconnected relationship that each dietary component can have with others.*

(Table 12.3). The nutrient profiles of the three dietary patterns are shown in Table 12.4; each pattern is nutrient-dense and was modeled to meet the DGAs including improving intakes of "nutrients of public health concern."

The 2015–2020 guidelines make primary recommendations in relation to the use of effective interventions at individual and population levels. We will discuss in the subsequent sections of this chapter strategies for obesity prevention, lifestyle management, and methods of effective intervention at individual and population levels. The DGAC brought forward the recommendations of the physical activity, lifestyle, and obesity reports.

IV EXPERT GUIDELINES ON NUTRIENT REQUIREMENTS FOR HEALTH PROMOTION AND CHRONIC DISEASE PREVENTION

In 2005, the National Heart Lung and Blood Institute (NHLBI) of the NIH recognized the need to update their guideline reports on CVD prevention and treatment. NHLBI convened stakeholder groups to guide this process and based upon their recommendations, expert panels were assembled to update and publish guidelines on overweight/obesity, cholesterol, and blood pressure (BP).

TABLE 12.3 Food Groups in the Three Dietary Patterns (Healthy US Style, Healthy Vegetarian, and Healthy Mediterranean-Style) Modeled by the DGAC[a]

Food Group[b]	Healthy US-Style Pattern	Healthy Vegetarian Pattern	Healthy Med-Style Pattern
Fruit	2 cups per day	2 cups per day	2½ cups per day
Vegetables	2½ cups per day	2½ cups per day	2½ cups per day
Legumes	1½ cups per week	3 cups per week	1½ cups per week
Whole grains	3 oz eq per day	3 oz eq per day	3 oz eq per day
Dairy	3 cups per day	3 cups per day	2 cups per day
Protein foods	5½ oz eq per day	3½ oz eq per day	6½ oz eq per day
Meat	12½ oz eq/week	–	12½ oz eq/week
Poultry	10½ oz eq/week	–	10½ oz eq/week
Seafood	8 oz eq/week	–	15 oz eq/week
Eggs	3 oz eq/week	3 oz eq/week	3 oz eq/week
Nuts/seeds	4 oz eq/week	7 oz eq/week	4 oz eq/week
Processed soy	½ oz eq/week	8 oz eq/week	½ oz eq/week
Oils	27 g per day	27 g per day	27 g per day

[a]*Intakes shown at the 2000 kilocalorie level as a percent of the goal or the limit for a 19- to 30-year old.*
[b]*1 cup = 240 mL; 1 ounce (oz) = 28 g.*

TABLE 12.4 Nutrients in the Three Dietary Patterns (Healthy US Style, Healthy Vegetarian, and Healthy Mediterranean-Style) Modeled by the DGAC[a]

Nutrient	Healthy US-Style Pattern (% Goal/Limit)	Healthy Vegetarian Pattern (% Goal/Limit)	Healthy Med-Style Pattern (% Goal/Limit)
Protein—% RDA	198	155	194
Protein—% Energy	18	14	18
Fat—% Energy	33	34	32
SFA[a]—% Energy	8	8	8
CHO—% RDA	197	211	199
CHO—% Energy	51	55	52
Fiber—% goal	109	126	112
Calcium—%RDA	127	133	100
Iron—%RDA	93	96	95
Vitamin D—%RDA	46	37	42
Potassium—%AI	71	70	71
Sodium[a]—%UL	76	61	72

[a]*Intakes shown at the 2000 kilocalorie level as a percent of the goal or the limit for a 19- to 30-year old.*

The published reports are collaboration between the ACC, the ACC [7], as well as TOS [6]. Because of the reports, focus on nutrition, physical activity, the guidelines on overweight and obesity, and lifestyle prevention and management of CVD will be summarized in this chapter.

A 2013 ACC/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults

1 Overview of Critical Questions Addressed by the Obesity Panel to Update Existing Guidelines

The Obesity Panel that was assembled in 2008 consisted of experts charged with updating existing NHLBI guidelines on overweight/obesity. Over a 5-year period, the panel reviewed and summarized high-quality, peer-reviewed published research spanning 1998 through 2011 in order to answer five critical questions (CQ1−CQ5):

- CQ1: Among overweight and obese adults, does weight loss produce CVD health benefits and what health benefits can be expected with different degrees of weight loss?
- CQ2: What are the CVD-related health risks of overweight and obesity and are the current cutpoints for overweight (BMI 25−29.9 kg/m^2), obesity (BMI >30 kg/m^2), and waist circumference (>102 cm (>40 in) in men and >88 cm (35 in) in women) appropriate for population subgroups?
- CQ3: Which dietary strategies are effective for weight loss?
- CQ4: What is the efficacy/effectiveness of a comprehensive lifestyle intervention program (i.e., diet, physical activity, and behavior therapy) in facilitating weight loss or maintaining weight loss?
- CQ5: What is the efficacy and safety of bariatric surgery? What is the profile (BMI and comorbidity type) of patients who might benefit from surgery for obesity and related conditions?

The panel summarized research studies using evidence tables that were extensively analyzed and discussed in order to guide the panel's conclusions and recommendations. Each recommendation was graded on the quality of the evidence base using standardized criteria. The Obesity Panel then created a treatment algorithm [16] which summarized its major recommendations and was intended to help direct practitioners in their assessment of patient risk and guide their management of overweight and obesity, and thereby achieve "best practices" in clinical and public health settings. Table 12.5 summarizes the major recommendations.

2 Findings and Recommendations From the Obesity Panel About Successful Weight Loss Approaches

As outlined in the treatment algorithm (ACC/ACC/TOS commentary), the Obesity Panel developed recommendations on the methods and frequency of assessing individual risk of overweight and obesity and then showed how to approach the design and implementation of effective weight loss and maintenance strategies. Their extensive literature review identified 15 distinct dietary regimens (e.g., low calorie, low-fat; low calorie higher protein; low calorie, low carbohydrate, etc.) that were shown to be sound and effective for weight loss management [16]. Of note in the panel's report is that all of the identified dietary regimens were shown to be equally effective in promoting weight loss *as long as* calories were controlled [16]. The report thus emphasizes that current research supports a wide array of effective dietary intervention options for weight loss management.

The panel recommended that weight loss be initiated in obese individuals and overweight persons with elevated CVD risk or other obesity-related comorbidities. According to the 2015−2020 DGAs, over half of adult Americans currently meet these criteria. They also recommended that patient's "readiness to change" be assessed and, as appropriate, that strategies be matched to the individual's health needs and lifestyle characteristics in order to establish a sound, *personalized* approach to weight management. It was noted that evidence-based, web and mobile tools and other technologies were emerging to enhance the effectiveness of weight loss and weight maintenance strategies.

The Obesity Panel report also points out that comprehensive lifestyle interventions, particularly approaches that incorporate dietary behavior change and physical activity, are the "gold standard" for weight loss management and weight loss maintenance. The panel used expert opinion to recommend the use of multidisciplinary teams for comprehensive lifestyle interventions, if available, or referral to nutrition professionals for dietary counseling and weight loss management.

3 Findings and Recommendations From the Obesity Panel About the Intensity and Duration of Weight Loss Approaches

The panel also examined the evidence on realistic, quantitative weight loss outcomes and made recommendations on the intensity and duration of treatment. Dietary interventions alone as well as high intensity comprehensive lifestyle programs (typically integrating diet, physical activity, and behavioral therapy) were found to result in weight losses in adult men and women at 6 months of up to 26 lb (12 kg) on average (ranging from 9 to 26 lb (4−12 kg))

TABLE 12.5 Major Recommendations for Management of Overweight and Obesity in Adults: 2013 AHA/ACC/TOS Guidelines [61]

1. *Identify Patients Who Need to Lose Weight*
   - Measure height and weight and calculate BMI at annual visits or more frequently.
   - Use the current overweight (BMI >25.0–29.9 kg/m^2) and obesity (BMI ≥30 kg/m^2) to identify adults who may be at elevated risk of CVD and the current cutpoint for obesity (BMI ≥30 kg/m^2) to identify adults who may be at elevated risk of mortality from all causes.
   - Advise overweight and obese adults that the greater the BMI, the greater the risk of CVD, type 2 diabetes, and all-cause mortality.
   - Measure waist circumference at annual visits or more frequently in overweight and obese adults. Advise adults that the greater the waist circumference, the greater the risk of CVD, type 2 diabetes, and all-cause mortality. Use the cutpoints currently in common use (from either NIH/NHLBI [>102 cm (>40 in) in men or >88 cm (>35 in) in women] or WHO/IDF [>94 cm (37 in) in men and >80 cm (31.5 in) in women]) to identify patients who may be at increased risk until further evidence becomes available.
2. *Match Treatment Benefits with Patients' Risk Profiles*
   - Counsel overweight and obese adults with CVD risk factors (high BP, hyperlipidemia or hyperglycemia) that lifestyle changes that produce even modest, sustained weight loss of 3–5% (of body weight) produce clinically meaningful health benefits, and greater weight losses produces greater benefits.
3. *Use Evidence-Based Dietary Strategies for Weight Loss*
   - Prescribe a diet to achieve reduced-calorie intake for overweight or obese individuals who would benefit from weight loss, as part of a comprehensive lifestyle intervention. Any one of the following methods can be used to reduce food and calorie intake:
     - Prescribe 1200–1500 kcal/day for women and 1500–1800 kcal/day for men (kilocalorie levels are usually adjusted for the individual's body weight);
     - Prescribe a 500 kcal/day or 750 kcal/day energy deficit; or
     - Prescribe one of the (identified 15) evidence-based diets that restrict certain food types (such as high-carbohydrate foods, low-fiber foods, or high-fat foods) in order to create an energy deficit by reduced food intake.
   - Prescribe a calorie-restricted diet, for overweight and obese individuals who would benefit from weight loss, based on the patient's preferences and health status and refer to a nutrition professional (e.g., RD) for counseling. A variety of dietary approaches can produce weight loss in overweight and obese adults.
4. *Use Evidence-Based Comprehensive Lifestyle Interventions, if Possible*
   - Advise overweight and obese individuals who wish to lose weight to participate for ≥6 months in a comprehensive lifestyle program that assists participants in adhering to a lower calorie diet and in increasing physical activity through the use of behavioral strategies. In overweight and obese individuals, in-person comprehensive lifestyle interventions consisting of diet, physical activity, and behavior therapy provided by a skilled professional team of nutritionists, exercise specialists, and behaviorists in a group or individually, have shown the highest success.
   - Prescribe on-site (face-to-face), high intensity (i.e., ≥14 sessions in 6 months) comprehensive weight loss interventions provided in individual or group sessions by a trained interventionist (e.g., RDs, psychologists, exercise specialists, health counselors, or professionals in training).
   - Recognize that electronically delivered weight loss programs (including by telephone) that include personalized feedback from a trained interventionist (e.g., RDs, psychologists, exercise specialists, health counselors, or professionals in training) can be prescribed for weight loss but may result in smaller weight loss than face-to-face interventions.
   - Utilize commercial-based programs that provide a comprehensive lifestyle intervention as an option for weight loss, provided there is peer-reviewed published evidence of their safety and efficacy.
   - Use a very low calorie diet (defined as <800 kcal/day) only in limited circumstances and only when provided by trained practitioners in a medical care setting where medical monitoring and high intensity lifestyle intervention can be provided. Medical supervision is required because of the rapid rate of weight loss and potential for health complications.
   - Advise overweight and obese individuals who have lost weight to participate, when possible, in a long-term (≥1 year) comprehensive weight loss maintenance program. For weight loss maintenance, prescribe face-to-face or telephone-delivered weight loss maintenance programs that provide regular contact (monthly or more frequent) with a trained interventionist (e.g., RDs, psychologists, exercise specialists, health counselors, or professionals in training) who help participants engage in high levels of physical activity (i.e., 200–300 minutes/week), monitor body weight regularly (i.e., weekly or more frequent), and consume a reduced-calorie diet (needed to maintain a lower body weight).
5. *Use Appropriate Methods to Selecting Patients for Bariatric Surgical Treatment for Obesity*
   - Advise adults with a BMI ≥40 or (those who present with a) BMI ≥35 (and) with obesity-related comorbid conditions who are motivated to lose weight and who have not responded to behavioral treatment with or without pharmacotherapy with sufficient weight loss to achieve targeted health outcome goals that bariatric surgery may be an appropriate option to improve health and offer referral to an experienced bariatric surgeon for consultation and evaluation.
   - Advise individuals with a BMI <35 that there is insufficient evidence to recommend for or against undergoing bariatric surgical procedures.
   - Advise patients that choice of a specific bariatric surgical procedure may be affected by patient risk factors, including age, severity of obesity/BMI, obesity-related comorbid conditions, other operative risk factors, risk of short- and long-term complications, behavioral and psychosocial factors, and patient tolerance for risk as well as provider factors (surgeon and facility).

Source: Adapted from B.E. Millen, D.M. Wolongevicz, C.A. Nonas, A.H. Lichtenstein, 2013 American Heart Association. American College of Cardiology/The Obesity Society Guidelines for the management of overweight and obesity in adults: implications and new opportunities for registered dietitian nutritionists, J. Acad. Nutr. Diet. 114 (2014) 1730–1735 [61] and B.E. Millen, D.M. Wolongevicz, J.M. De Jesus, C.A. Nonas, 2013 American Heart Association. American College of Cardiology Guideline on lifestyle management to reduce cardiovascular risk: practice opportunities for registered dietitian nutritionists, J. Acad. Nutr. Diet. 114 (2014) 1723–1729 [62].

and from 9 to 22 lb (4−10 kg) after 12 months. While weight regain occurred as treatments continued for longer duration (up to 2 years or longer), average longer-term weight losses were sufficient to recommend continued weight management treatment by qualified professionals. The panel determined that weight losses of 5−10% of body weight at 6 months are feasible with diet alone and more intensive interventions. Nonetheless, sustained weight losses of 3−5% of body weight were identified as feasible and as sufficient to improve CVD and diabetes risk factor profiles in overweight and obese adults (with and without diabetes); greater weight losses were associated with even better outcomes.

The impact of weight loss on CVD- and diabetes-related risks was also seen to be improved by medical nutrition therapy protocols that targeted risk factors (e.g., lower SFA protocols provided additional benefits on low-density lipoprotein (LDL)-cholesterol). High intensity, on-site comprehensive interventions (≥14 sessions in 6 months) were recommended for weight loss management and these recommendations align well with the most recent US Public Services Task Force [17] and Centers for Medicare and Medicaid Services recommendations [18]. The overall effectiveness of group or individual treatment regimens was similar so both are recommended as long as conducted on-site by trained interventionists (registered dietitians/registered dietitian nutritionists (RDs/RDNs), exercise specialists, and behaviorists). Web- and telephone-based interventions offered in academic and healthcare settings were considered as emerging areas with future potential but to be generally less effective than in-person treatments.

In summary, the Obesity Panel found that there is abundant current high-quality research evidence on the effectiveness of calorie-restricted dietary regimens and comprehensive lifestyle interventions for weight loss management in adult men and women. The panel provided guidelines on the assessment of risk for overweight and obesity in adults and recommended that individuals who would benefit from weight loss be referred to comprehensive lifestyle intervention implemented by trained interventionists (including RDs/RDNs, exercise specialists, and behavioral strategies) when available, and to the RD/RDN for dietary counseling for weight loss and medical nutrition therapy for CVD risk factor management. Referral to other modes of weight management including commercial programs and web and telephonic counseling by trained interventionists was recommended by expert opinion *but only if* scientific evidence of efficacy is available and with the acknowledgment that the amount of weight lost might be lower than with comprehensive lifestyle interventions implemented by trained interventionists. The 2015−2020 DGAC brought forward the recommendations of the Obesity Panel.

B 2013 ACC/AHA Guideline on Lifestyle Management to Reduce Cardiovascular Risk

1 Overview of CQs Addressed by the Lifestyle Workgroup to Develop Recommendations for CVD Risk Management Using Behavioral Intervention Strategies

Concurrent with the establishment of expert panels in 2008, the NHLBI also established a Lifestyle Workgroup to develop recommendations for CVD risk management using behavioral intervention strategies. This workgroup served alongside and complemented the ongoing guideline updates on overweight/obesity (discussed above), cholesterol, and BP. The Lifestyle Workgroup focused primarily on nutrition and physical activity, topics determined to be most relevant to the clinical practice of prevention and treatment of abnormal lipids and high BP. Over a 5-year period, the Lifestyle Workgroup set out to answer three overarching CQs:

> CQ 1: Among adults (18 years and older), what is the effect of dietary patterns and/or macronutrient composition on CVD risk factors, when compared to no treatment or to other types of interventions?
> CQ2: Among adults, what is the effect of dietary intake of sodium and potassium on CVD risk factors and outcomes, when compared to no treatment or to other types of interventions?
> CQ3: Among adults, what is the effect of physical activity on BP and lipids when compared to no treatment or to other types of interventions?

To answers these broad questions and their detailed components, the workgroup reviewed high-quality published literature consisting of systematic reviews, RCTs, and observational studies that included hard endpoints as outcomes, which spanned the time frame from 1998 through 2009. The RCTs examined by the Lifestyle Workgroup were limited to those in which research participants' body weights were kept stable so as to determine the impact of dietary patterns alone or in conjunction with physical activity on health-related risks and outcomes under participants' weight-stable conditions. The impact of change in body weight on health outcomes was a specific focus of the Overweight/Obesity Guideline that was reviewed earlier [6].

In 2012, the NHLBI partnered with the ACC and AHA to adopt and publish the 2013 Guideline on Lifestyle Management to Reduce Cardiovascular Disease Risk [7]. The guidelines are meant to provide clinicians with recommendations on the most effective diet and physical activity strategies to manage dyslipidemia and hypertension to promote cardiovascular health.

2 Scientific Approach of the Lifestyle Workgroup

The Lifestyle Workgroup specifically reviewed the effect of dietary patterns, nutrient intakes, and physical activity in modifying CVD risk [19–23] and their relationship to CVD outcomes [24–32]. RCTs were also used to assess the efficacy [33–35] and effect of dietary pattern interventions (DASH and Mediterranean-style (MED) patterns). In addition, published systematic reviews (i.e., qualitative reviews and meta-analyses) of controlled feeding studies were used to evaluate how replacement of energy from SFA and *trans*-fat with alternative energy sources (carbohydrates or monounsaturated (MUFA) or polyunsaturated (PUFA) fats) impacts levels of LDL-C and high density lipoprotein (HDL-C) cholesterol and triglycerides. The influence of dietary sodium on BP was assessed in the DASH-Sodium trial [36,37]. Observational cohort investigations were used to evaluate relationships between selected nutrients (sodium and potassium) and hard endpoints (e.g., stroke, CVD mortality) although, ultimately, potassium was omitted from the Workgroup's recommendations because evidence was insufficient to determine the effect of dietary intake of potassium on BP on CVD outcomes (including stroke). The effect of physical activity on CVD risk factors and outcomes was evaluated using systematic reviews only [38–40]. Of note in this report is that observational studies included study participants with varying health profile characteristics whereas those involved in RCTs typically were relatively "high-risk" individuals with established CVD, diabetes or hypertension, or elevated CVD risk. The reader should be aware of how this evidence-base may influence the generalizability of recommendations made by the panel.

3 Summary of Recommendations from the Lifestyle Workgroup

The Lifestyle Workgroup used a public health model and articulated recommendations at both the population and individual levels as summarized in Table 12.6. The Lifestyle Workgroup's guidelines emphasize the benefits of healthy lifestyles for CVD risk reduction and prevention in populations as well as individuals. The guidelines for individuals focus on those who would benefit from BP and LDL-C lowering. The workgroup recommended use of the 2013 ACC/AHA Blood Cholesterol Treatment Guideline [41] for further guidance identifying individuals with elevated CVD risk profiles and for information on drug treatment for cholesterol lowering. In addition, they suggested referring to the Scientific Advisory on High Blood

TABLE 12.6 Lifestyle Workgroup's Lifestyle Management Guideline Recommendations [62]

Population-Level Recommendations

The Workgroup recommended that all adult American men and women consider the following:

- Consume a heart healthy) dietary pattern that emphasizes intake of vegetables, fruits, and whole grains; includes low-fat dairy products, poultry, fish, legumes, nontropical vegetable oils and nuts; and limits intake of sodium, sweets, sugar-sweetened beverages, and red meats.
  - Adapt this (heart healthy) dietary pattern to appropriate calorie requirements, personal and cultural food preferences, and nutrition therapy for other medical conditions (including diabetes mellitus).
  - Achieve this (heart healthy) pattern by following plans such as the DASH dietary pattern, the USDA Food Pattern, or the AHA Diet.
- Engage in 2 h and 30 min a week of moderate-intensity, or 1 h and 15 min (75 min) a week of vigorous-intensity aerobic physical activity, or an equivalent combination of moderate- and vigorous-intensity aerobic physical activity.
  - Aerobic activity should be performed in episodes of at least 10 min, preferably spread throughout the week.

The Workgroup recommended that all adults engage in nutrition and physical activity behaviors consistent with the DGAs (which was current at the time of the publication of their report) and the 2008 Physical Activity Guidelines for Americans.Achieve and maintain a healthy weight. Refer to the 2013 Overweight and Obesity Guideline for recommendations on weight loss and maintenance.

Individual-Level Recommendations

Advise adults who would benefit from LDL-C or BP lowering to:

- Consume a dietary pattern as described in the Population Recommendations above for heart health. The report further states "Dietary planning and nutritional counseling is often facilitated by referral to a nutrition professional," defined as a RD/RDN.

Advise adults who would benefit from LDL-C lowering to:

- Aim for a dietary pattern that achieves 5–6% of calories from SFA.
- Reduce percent of calories from SFA.
- Reduce percent of calories from *trans*-fat.

Advise adults who would benefit from BP lowering to:

- Lower sodium intake
- Consume no more than 2400 mg of sodium/day
  - Further reduction of sodium intake to 1500 mg/day is desirable since it is associated with even greater reductions in BP and
  - Even without achieving these goals, reducing sodium intake by at least 1000 mg/day lowers BP.
  - Combine the DASH dietary pattern with lower sodium intake.

In general, advise adults to engage in aerobic physical activity to reduce LDL-C, non-HDL-C, and BP: 3–4 sessions a week, lasting on average 40 min per session, and involving moderate-to-vigorous intensity physical activity.

Pressure Control from the AHA, ACC, and CDC [42] which recommends lifestyle management in the treatment of all adults with hypertension (BP: >140 mmHg/ >90 mmHg).

The Workgroup's report also provided food-based recommendations associated with the dietary patterns that reflect the highest overall benefits on CVD risk. It was noted that the recommended "heart healthy" dietary patterns can be achieved by using multiple approaches [13,43,44].

In summary, the guidelines on lifestyle management emphasize the benefits of behavior change, particularly those that facilitate healthy dietary patterns and physical activity, for population and individual CVD risk reduction and health promotion. The guidelines are largely consistent with the multiple healthy dietary patterns defined in the 2015–2020 DGAs [12] as well as the recommendations in the then current 2010 Dietary Guidelines [44] and the 2008 Physical Activity Guidelines for Americans [45]. The reader is directed to the full report [46] for further details on the studies reviewed and the expected impact of dietary pattern and physical activity interventions on BP and lipid outcomes. The 2015–2020 DGAC brought forward the recommendations of the AHA/ACC Lifestyle Workgroup.

V USING NUTRITION POLICIES AND EXPERT GUIDELINES TO INFORM THE PROFESSIONAL PRACTICE OF NUTRITION

Professional practice of nutrition is influenced in many ways by nutrition policies and expert guidelines. For example, as noted above, the *DGAs* are not only used within the agencies that fall in the very broad jurisdictions of HHS and USDA, but also to inform professional practice and can be influential in changing the food and physical activity environment. Despite the wide reach of the guidelines and the strong and growing body of literature showing that when individuals are adherent to healthy, nutrient-dense dietary patterns (and physical activity) as advocated in the DGAs, they have lower risk of overweight and obesity [47], smaller waist circumference [47,48], lower insulin resistance [49], reduced odds of carotid atherosclerosis [50], slower progression of carotid atherosclerosis [51], and lower total and CVD mortality [52], most Americans are not adherent.

Adherence to dietary guidance that promotes health has been an ongoing challenge in the United States for decades, and contributing to the prevalence of preventable diet-related conditions that have increased or plateaued at extremely high levels. To address this critical concern, multiple stakeholders such as Congress, federal agencies, and private foundations have created initiatives to promote programs and policies to achieve healthful dietary patterns in Americans. One organization that holds a unique position to address the science underlying dietary policy is the National Academies of Science, Engineering, and Medicine. It is independent from government and free from influence from political and special interest groups, and as such, the Academies can provide complementary guidance on nutrition issues. The National Academies were established by Congress, over a century ago, and incorporate four organizations: The National Academy of Sciences, the National Academy of Engineering, the Institute of Medicine (IOM), and the National Research Council. Over the past two decades, the IOM's Food and Nutrition Board and the Board on Population Health and Public Health Practice have overseen multiple consensus reports [53,54] and workshops [55–60] that address one or more aspects of the complexities of promoting adherence to *DGAs* through professional practice.

One such example is a report by the Food and Nutrition Board's committee on "*Accelerating Progress in Obesity Prevention*" [58]. It gives accessible and useful information for professional practice. The report addresses the need for improved communication of the threats of obesity and the importance of involving multiple sectors in addressing it. The report also concludes that many interest groups, including healthcare providers, policy makers, the general public, those who make decisions about food environments (including food product composition and access), businesses, private sectors, and educators, need to pool efforts and collaborate in order to address the obesity epidemic at individual and population levels. The report outlines strategies that can be used in healthcare environments that ensure incentives for, access to, coverage of routine obesity prevention, screening, diagnosis, and treatment services. Furthermore, it acknowledges that it is necessary to empower communities of individuals to work toward changes in their food environments that support efforts to achieve healthy lifestyles for healthy weight. In a workshop report from this committee in 2012, food and nutrition policies and environments were discussed [53]. The report documents several issues that may impact professional practice and adherence to the DGAs and other expert dietary guidelines. First, a key factor affecting weight and health is the availability of healthy and unhealthy foods, particularly in areas that are densely populated with fast-food outlets or have limited retail outlets with fresh and nutrient-dense food product offerings. Many communities lack nutrition policies or standards to guide what is available in neighborhoods, schools, or workplaces. Second, community-wide programs can promote healthier choices and can support changing behaviors at individual and population levels. Community environments encompass

the types and locations of restaurants and supermarkets and their accessibility within a particular community. The consumer environment is defined as what is encountered when a consumer goes out to eat or to purchase food in a restaurant, school, or elsewhere. The report also addresses factors such as the availability of healthful food choices within the environment; availability of nutrition information, pricing, and product placement; and the need to measure the food environment and policies in schools, worksites, food stores, restaurants, and local/state communities. Lastly, it summarizes the ideas shared during the workshop regarding implementing surveillance of the food environment to track nutrition-related environmental and policy changes and their effects to complement the current focus on food and obesity at the individual level.

Further support for professional practice can be found in a report by the committee on Prevention of Obesity in Children and Youth which was convened by the Food and Nutrition Board in collaboration with the IOM's Board on Health Promotion and Disease. In developing a prevention-focused action plan to decrease the prevalence of obesity in children and youth in the United States, the committee's report resonates with the DGAs and other IOM reports and addresses the importance of making changes at many levels and in numerous environments involving multiple stakeholders from diverse segments of society. The committee describes an environmental-behavioral synergy with multiple prevention strategies including improving the proportion of children meeting *DGAs* and promoting a healthy diet of appropriate quality and quantity. It also describes interim policy and system changes that are needed such as improving access to and affordability of fruits and vegetables for populations with low incomes, increased numbers of new industry products and advertising messages that promote energy balance at a healthy weight, increased availability and affordability of healthful foods and beverages at supermarkets, grocery stores, and farmers markets located within walking distance of the communities they serve; and changes in institutional and environmental policies that promote energy balance.

The IOM's Board on Population Health and Public Health Practice's committee on Public Health Priorities to Reduce and Control Hypertension in the US population reviewed available public health strategies specifically for reducing and controlling hypertension in the US population, including both science-based and practice-based knowledge. The committee concluded that a stronger focus is needed on primary prevention of hypertension and underscored the important role of nutrition-related factors in the prevention of hypertension and the reduction of risk factors for hypertension [54]. For example, the committee recommended collaboration across CDC units and their external partners to ensure that population-based lifestyle and behavioral interventions, such as promotion of consumption of a healthy diet through community-messaging and improving access to nutrient-dense foods, are delivered in a coordinated manner; and the interventions should include an evaluation of feasibility and impact.

Additionally, proceedings from workshops of the standing committee on Childhood Obesity Prevention detailed nutrition-related challenges and opportunities for change in marketing to children and youth [56] and identified allies for obesity prevention and common ground for dialog among them [57]. Both reports commented on the need for addressing both personal responsibility of healthy nutrition and lifestyle behaviors through evidenced-based nutrition and lifestyle interventions and environmental change.

a. In the summary report from the workshop on "*Alliances for Obesity Prevention: Finding Common Ground*," a diverse group of potential allies were identified that cut across sectors and may be unexpected and unlikely. It is noted that while parents have a responsibility in the nutrition-related aspects of childhood obesity epidemic, they also need support to parent effectively and the environment needs to be modified to make it easier for them to protect their children from the threats of obesity [57].

b. In the summary report from the workshop on "*Challenges and opportunities for change in food marketing to children and youth*," in trying to get change in food marketing to children, industry partners are needed to lower the high level of exposure children have to abundant commercial advertising and marketing for food. It is suggested that food and beverage companies use creativity, resources, and marketing practices to promote and support more healthful diets and meals [56]. The objective of these recommendations is to promote product development of healthy foods; product reformulations of unhealthy ones; expansion and promotion of healthier meals; and provision of nutrition information at the point of choice and consumption. Ultimately, efforts on the part of food industry are needed to ensure that the guidelines can ultimately promote and maintain health.

c. A workshop which included representatives from food industry, academia, and government discussed how food technology can be leveraged for obesity prevention and reduction efforts [55]. In this workshop's summary report, it was documented that food technologies have been used to find sugar substitutes, reduce fat and energy density of foods, decrease portion size, and increase micronutrient intake. However, it was

suggested that there is no "magic bullet," and taste and affordability is important to consumers making it important to educate consumers and create partnerships between industry and government to develop novel food products for commercialization.

There is remarkable consistency, across the Food and Nutrition Board's reports, in the conclusions that state and local public health jurisdictions integrate evidence-based nutrition guidelines into programmatic efforts toward systems, environmental, and policy changes that will support healthful eating and lifestyles at individual and population levels. Ongoing efforts are also needed to create recommendations for future action and to measure indicators of progress in implementing actions. These reports have served as roadmaps to solutions in professional practice, and are a resource for many practitioners.

A public health approach, addressing both individual and population approaches, is strongly advocated in the *2015–2020 DGAs* with recommendations that everyone has a role in creating and supporting healthy eating patterns in multiple settings nationwide, from home to school to healthcare and public health systems, to worksites to communities; and including public–private partnerships and addressing eating environments that are conducive to overconsumption. This is supported in an IOM report emphasizing that broad-based public health strategies have been impactful in public health efforts such as automobile safety and reducing tobacco use [1].

VI SUMMARY

Diet not only is related to the prevention and treatment of many chronic diseases, but also demonstrates the efficacy and impact of preventive nutrition and lifestyle behavioral interventions on a wide range of health outcomes. Achieving a dietary pattern that is healthful in quality and quantity and increasing the proportion of the population who meet the *DGAs* will require public health approaches that are designed to reach individuals as well as entire communities. Evidence-based approaches that address the needs and preferences of individuals and communities served are critical. In addition, every level of the socio-ecological model will need to be addressed and collaborations will be needed at the local, state, and national levels to change individual behavior and enhance food environments. Partnerships across sectors will be needed to reach the public with preventive and therapeutic nutrition services and to make it as easy as possible to adopt healthy nutrition and lifestyle behaviors. Nutrition policies and expert guidelines serve an important role in improving diet and ultimately advancing health at individual and population levels.

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Chapter 13

Nutritional Recommendations for Athletes

Sara C. Campbell and Paul J. Wisniewski

Rutgers, The State University of New Jersey, New Brunswick, NJ, United States

I INTRODUCTION

Successful performance in sport is a result of many different factors. Arguably one of the most important factors is genetic predisposition; however, meeting optimal energy requirements is also critical for performance. Athletes who make poor food choices may prevent themselves from achieving their optimal potential. There is a strong evidence base that has established a role for optimal dietary strategies to enhance performance [1]. This begins with the recognition that when performing regular exercise, hard physical labor, or exercise training, utilization of carbohydrate (CHO), fat, and protein to make energy increases. As a result, the requirements of macro- and micronutrients increase.

The relationship between nutrition and physical performance has fascinated people for a long time. It has become clear that different types of exercise and different sports have different energy and nutrient requirements, and therefore food intake must be adjusted accordingly. It has been clearly shown that certain nutritional strategies can enhance performance, improve recovery, and result in more profound training adaptations. However, the diets of athletes are often reported as nutritionally inadequate [2]. The knowledge an athlete possess on proper nutrition, which is typically greater than that of the general public, is still lacking and mostly based on coach-driven advice and may be influenced by financial constraints [3]. The role of the sports nutritionist becomes increasingly critical to correct dietary inadequacies and promote health by creating a positive impact on the optimal performance of the athlete [4]. Nutrition strategies should be aimed at modulating training-induced adaptations, most important in muscle. This chapter reviews the nutritional demands of exercise in relation to the physiological demands and examines strategies to fuel exercise performance.

II ENERGY REQUIREMENTS FOR ATHLETES

Adequate energy requirement is a delicate balance between the amount of food intake required to maintain total daily energy expenditure (TDEE) and body weight [5]. TDEE is the sum of resting metabolic rate (RMR 60−75%), diet-induced thermogenesis (DIT 10%), and thermic effect of exercise (TEE 15−30%). During exercise, the energy expenditure increases several-fold mostly as a result of skeletal muscle contraction. Therefore, depending on how much exercise an individual performs, TEE is by far the most variable component of TDEE. TEE includes all energy expended above the RMR and DIT. In addition, it is influenced by lifestyle activities, the nature of exercise performed, gender, and prior nutritional status. TEE can contribute from virtually nothing to more than 80% of energy expenditure. In highly trained, very active individuals, the TEE can amount to up to 8000 kcal per day. In sedentary people, the TEE may be as low as 100 kcal per day. Energy expenditure during physical activity ranges from 5 kcal/minute for very light activities to up to 25 kcal/minute for very high-intensity exercise (Table 13.1).

Because of the variable nature of TEE, it is imperative that athletes understand how much energy they use to perform their sport, which will be indicative of energy intake required to maintain this performance. Energy restriction in athletes has deleterious side effects because in most situations, substrate (CHOs, fats, and proteins) availability can become critical for the continuation of exercise.

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TABLE 13.1 Rough Estimation of Energy Expenditure in a Variety of Sports[a]

Activity Level	kJ/min	kcal/min	Examples
Resting	4	1	Sleeping, watching TV
Very light activities	12–20	3–5	Sitting and standing activities, driving, cooking, card playing, desk work, typing
Light activities	20–28	5–7	Walking (slowly), baseball, bowling, horseback riding, cycling (very slowly), gymnastics, golf
Moderate activities	28–36	7–9	Jogging, cycling (at a moderate pace), basketball, badminton, soccer, tennis, volleyball, brisk walking, swimming (at an easy pace)
Strenuous activities	36–52	9–13	Running (10–13 km/h), cross-country skiing, boxing, cycling (30–35 km/h), swimming, judo
Very strenuous activities	>52	>13	Running (>14 km/h), cycling (>35 km/h)

[a]*Energy expenditure depends on body mass, the intensity, and the duration of rest periods.*
Source: W.D. McArdle, F.I. Katch, V.L. Katch, Exercise Physiology: Energy, Nutrition and Human Performance, fifth ed., Lippincott, Williams & Wilkins, Philadelphia, PA, 2006.

These substrates and their availability for energy production during exercise must be obtained through nutrition. In endurance athletes, e.g., CHO depletion is one of the most common causes of fatigue. Therefore, adequate CHO intake is essential to prevent early fatigue as a result of CHO depletion.

A Energy Balance

Energy balance represents the difference between energy intake and energy expenditure. When the energy intake exceeds the energy expenditure, there is a positive energy balance, which results in weight gain. When the energy intake is below the energy expenditure, there is a negative energy balance and weight loss results. Over the long term, energy balance is maintained in weight-stable individuals, even though on a day-to-day basis this balance may sometimes be positive and sometimes negative. Although life is not always that simple, basic manipulation of the energy balance equation will yield weight loss or gain. For example, if someone wishes to lose weight, it is important to increase the energy expenditure relative to the energy intake or decrease energy intake below daily energy expenditure. The opposite would be true if an individual is trying to achieve weight gain.

B Energy Balance in Different Activities

Some physical activities require higher energy outputs than others, as shown in Table 13.1. Tennis, e.g., has relatively low energy expenditure if played recreationally. However, during a match the exercise can be intense, and energy expenditure during that short burst of exercise can be very high. However, because this is typically followed by a longer period of relatively low intensity (walking) or even standing, the average energy expenditure for this activity is relatively low. On the other hand, in continuous sports such as cycling and running, in which there is usually no recovery during the activity, energy expenditures can be relatively high.

Physically active individuals need to be aware of the energy input necessary to maintain weight and performance. The simplest way to do this is to calculate resting daily energy expenditure (RDEE): RDEE (kcal) = 370 + 21.6 (fat-free mass (FFM), kg). In order to calculate FFM, two simple steps must be followed. First, determine your weight in kilograms. This can be done by taking your body weight in pounds and dividing it by 2.2; e.g., if you weigh 220 lb, your weight in kilograms is 220/2.2 = 100 kg. Second, calculate FFM in kilograms. Take your weight in kilograms and multiply it by (1 − your body fat percentage). For example, if you weigh 100 kg and your body fat percentage is 12%, then your FFM = 100 kg × (1 − 0.12) = 88 kg. These simple calculations allow individuals to understand exactly what their energy requirements are so that they can match energy intake with expenditure. As stated previously, inadequate energy intake relative to energy expenditure can compromise performance and will negate the benefits of training. This is because limiting nutrient intake can result in loss of lean tissue mass that results in the loss of strength and endurance as well as compromising the immune, endocrine, and musculoskeletal systems.

Energy expenditure varies by type, frequency, intensity, and duration of the activity. The energy systems (Table 13.2) used during exercise will dictate expenditure. These energy systems are the immediate energy system (i.e., the ATP-PC system, phosphagen system, and power system), short-term energy system (i.e., anaerobic energy system, glycolytic system, and lactic acid system), and long-term energy system (i.e., aerobic energy system). The immediate energy system uses adenosine triphosphate

TABLE 13.2 Energy Systems and Energy Use

Energy System	Rate[a]	Capacity[b]
ATP-creatine phosphate	1.6–6.0	24
Anaerobic glycolysis	1.0–1.5	240
Aerobic		
CHO	0.5	3000
Fat	0.24	Unlimited

[a] *μmol/g muscle/s (power).*
[b] *μmol/g muscle (energy supply).*

TABLE 13.3 Sources of Energy for Exercise

Source	Storage Form	Total Body Calories (kcal)	Distance Covered
ATP	Tissues	1	17.5 yards
Creatine phosphate	Tissues	4	70 yards
CHO	Serum glucose	20	350 yards
Liver glycogen		400	4 miles
Muscle glycogen		1500	15 miles
Fat	Serum fatty acids	7	123 yards
Serum triglyceride		75	0.75 mile
Muscle triglyceride		2500	25 miles
Adipose tissue		80,000	800 miles
Protein	Muscle	30,000	300 miles

(ATP) and phosphocreatine to supply muscles with energy to do work, and exercises of this nature typically last only approximately 6–10 seconds so they are short, powerful, and high-intensity activities. Examples include the golf swing, tennis serve, and 40-yard dash. The short-term energy system is the anaerobic system, meaning that it does not require oxygen to produce energy. This system's primary substrate is CHO in the forms of glycogen and glucose, which is broken down via glycolysis. As no oxygen is being used for ATP production in this system, it results in the over accumulation of hydrogen ions in the muscle, thus lowering the pH (more acidic environment) and leading to a cessation in exercise due to the disruption of the glycolytic enzymes. This system can provide energy for approximately 60 seconds to 3 minutes, and some examples of exercises include a 440-m run or 100-m swim. Finally, the long-term energy system will provide fuel for exercise lasting longer than 3 minutes. This fuel is provided by muscle and liver glycogen; intramuscular, blood, and adipose tissue triglycerides; and a small amount of amino acids. This system requires oxygen for ATP production, and as it becomes available, oxidative pathways are used, which can produce large amounts of ATP for exercise events such as marathons, endurance cycling, and swimming. Because each energy system is supplied by various macronutrients, it is imperative that athletes eat a well-balanced diet that meets the recommendations. A summary of the macronutrients that are used to make ATP for muscular contraction is provided in Table 13.3.

1 What Is the Role of Body Composition?

Body weight and body composition are two factors that govern an athlete's decisions in food choices. Body weight may be an important influence on speed, endurance, and power, whereas body composition may influence strength, agility, and appearance. For example, a lean (more muscle than fat) appearance may be important for speed. In many sports in which body composition or body weight is believed to be important (gymnastics, dancing, body building, and weight category sports such as wrestling, judo, and boxing), athletes often try to maintain a negative energy balance in order to lose weight [6]. It is known that energy intakes in these sports can be very low [7,8] and this can be a detriment to performance.

At the other extreme are endurance or ultra-endurance sports, such as marathons, triathlons, and cross-country skiing, in which extremely high energy expenditures are known to occur that must be matched by appropriate calorie intake. Optimum performance in these sports depends on the individual maintaining energy balance on a day-to-day basis.

Females generally have lower energy intakes than males and often have lower intakes than would be necessary for being physically active [8,9]. For females, energy intake ranged from 5.1 MJ (1600 kcal) to 10.2 MJ (3200 kcal). For males, energy intake ranged from 12.1 MJ (2900 kcal) to 24.7 MJ (10,500 kcal). These differences in energy intake may be related to body size and weight, body composition, and the training volume. Team sport athletes have a moderate energy intake, whereas some of the endurance sports have been characterized by very high energy intakes. In fact, energy intakes in excess of 12.6 MJ (3000 kcal) for females and 16.7 MJ (4000 kcal) for males were reported only in endurance sports.

In summary, an individualized assessment of the athlete's body composition is warranted to determine an optimal competitive body weight and relative fatness, with the goal to optimize the athlete's health and performance. In addition, particular attention should be paid to female athletes, who are frequently known to consume less calories, and sports in which weight is of particular importance for competition.

2 How Do Athletes Assess Energy Cost of Activity?

As mentioned previously, one can use the RDEE equation to calculate resting energy requirements; however, there is also a need to understand how much energy is being used during exercise. There are several ways to accomplish this, including both direct and indirect means of measurement. To directly measure energy cost, one would use a human calorimeter. Although use of a human calorimeter is the most accurate way to assess energy cost, few facilities have one of these and it is not the most convenient or practical means of measurement. As such, indirect calorimetry has become the standard of measurement for this purpose. These measurements can be obtained by placing the subject on a metabolic cart, which can measure the amount of oxygen consumed and carbon dioxide produced. These values can then be used to calculate the respiratory quotient (RQ). Each macronutrient has its own RQ value. It is important to note that the RQ for protein is approximated at 0.80; however, an equation for protein is typically more convoluted and mostly unavailable compared to those for fats and CHOs. This is because proteins are not simply oxidized to carbon dioxide and water during energy metabolism. During metabolism, the protein is deaminated and the nitrogen and sulfur are excreted. The resulting keto fragments are then oxidized. All of this complicates things; however, the actual contribution of protein to energy metabolism is very low. For example, see the following equations:

$$\text{Respiratory quotient (RQ)} = \frac{\text{CO}_2 \text{ produced}}{\text{CO}_2 \text{ consumed}}$$

Examples of macronutrient RQ calculations.
CHO RQ calculation:

$$\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 = 6\text{CO}_2 + 6\text{H}_2\text{O} + 38 \text{ ATP (CHO)}$$
$$\text{RQ} = 6\text{CO}_2/6\text{O}_2 = 1.0 = 5.05 \text{ kcal/lO}_2$$

Fat RQ calculation:

$$\text{C}_{16}\text{H}_{32}\text{O}_2 + 23\text{O}_2 = 16\text{CO}_2 + 16\text{H}_2\text{O} + 129 \text{ ATP (fat)}$$
$$\text{RQ} = 16\text{CO}_2/23\text{O}_2 = 0.74.68 \text{ kcal/lO}_2$$

Additional indirect means of measurement include doubly labeled water (deuterium), heart rate monitors, accelerometers, or activity tracking by keeping daily activity logs. During activity tracking, each activity is given a metabolic equivalent (MET) that corresponds to how many times resting level the activity corresponds to. For example, an activity that is 6 METs would be six times resting metabolism. A full list of activities and their MET values are found in the "Compendium of Physical Activities" [10].

III MACRONUTRIENT RECOMMENDATIONS FOR ATHLETES

The previous sections provided a summary emphasizing the importance of proper nutrition with the understanding that this nutrition is what ultimately provides one with the substrate necessary to make ATP for muscular contraction (i.e., work). The remaining sections of this chapter summarize the pertinent literature related to appropriate recommendations for macro- and micronutrient intake.

A Carbohydrate

CHO fuel plays a major role in the performance of many types of exercise and sport. The depletion of body CHO stores is a cause of fatigue or performance impairments during exercise, particularly during prolonged (>90 minutes) sessions of submaximal or intermittent high-intensity activity (Fig. 13.1). Unfortunately, total body CHO stores are limited and are often substantially less than the fuel requirements of the training and competition sessions undertaken by athletes. Maximizing glycogen stores in both liver and muscle prior to the exercise bout is essential for optimal performance. Studies have shown that after exercise, muscle

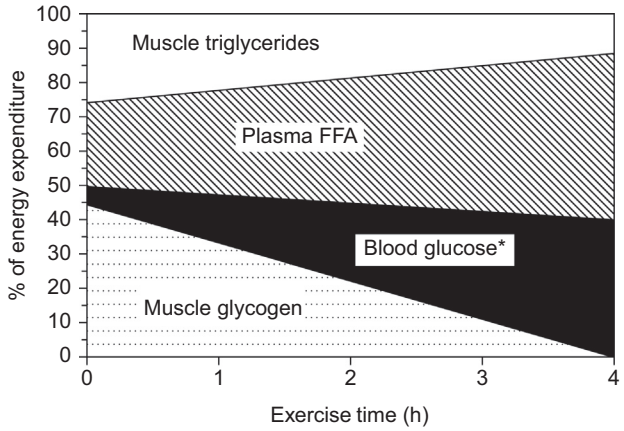

FIGURE 13.1 Percentage of energy derived from the four major substrates during prolonged exercise at 65–75% VO_2 max. The asterisk designates the importance of blood glucose contributing as a source of CHO energy for muscle as exercise is prolonged. Reproduced with permission from E.F. Coyle, Substrate utilization during exercise in active people, Am. J. Clin. Nutr. 61 (Suppl.) (1995) S968–S979.

TABLE 13.4 GI of Select Foods

High GI (>85)	Medium GI (60–85)	Low GI (<60)
Glucose	Banana	Fructose
Sucrose	Oatmeal	Apple
Bread	Pasta	Lentils
Potatoes	Rice	Milk
Sports drinks	Corn	Yogurt

glycogen stores can be returned to normal resting levels (350–500 mmol/kg dry weight muscle) with 24–36 hours of rest and an adequate CHO intake (6–10 g/kg body weight per day) [1,11,12]. Therefore, strategies for athletes include consuming CHO before, during, and in the recovery period between prolonged exercise bouts.

1 Preexercise CHO Intake

Feeding prior to exercise should take into account the timing of the meal prior to exercise, the intensity of the exercise that will be performed, and the type of CHO ingested—i.e., the glycemic index (GI) of the food and whether it is a solid or liquid. GI is assessed by how rapidly blood glucose levels rise after a fixed amount of ingested CHO. The GI of some foods can be found in Table 13.4.

Studies examining preexercise feedings have focused on various times prior to the bout and types of CHO given. Early research [13] examined feeding glucola (glucose solution), milk, or water 30 minutes prior to exercise at 80% maximal oxygen uptake (VO_2 max). Results of this study showed that glucola participants could not exercise for as long because of the stimulated insulin response to the skeletal muscle glucose uptake. This early study raised some issues about potentially restricting CHO consumption prior to competition so that exercise hypoglycemia could be avoided. Later findings, however, do not fully support this early study. Although research has consistently found clear metabolic differences in response to the timing of preexercise CHO ingestion within the hour before exercise, the performance effects have been somewhat equivocal. With the exception of the Foster study [13], research has either found no performance effects [14–21] or a performance improvement [22–24]. Based on current research, it appears that there is little evidence to suggest benefits from avoiding CHO intake in the hour before exercise. If one is concerned that he or she might be susceptible to hypoglycemia during exercise, CHO can be ingested just prior to exercise (in the last 5 minutes), or foods that have a low GI can be consumed. This can be done to minimize the risks of hypoglycemia because as exercise begins and the glucose transporter-4 (GLUT-4) is stimulated to translocate to the cell membrane, it will be responsible for bringing in glucose to the cell for energy metabolism. To support this idea, Thomas et al. [25] fed participants glucose, potato, lentils, or water 60 minutes prior to exercise at 75% VO_2 max. Results showed that blood glucose and insulin response was higher in the glucose and potato groups compared to the lentil group. Finally, a large body of work has shown that the intake of a substantial amount of CHO (~200–300 g) 3 or 4 hours before exercise enhances various measures of exercise performance compared to performance undertaken after an overnight fast [26–28].

The form of CHOs ingested—solid versus liquid—before exercise has also been examined for its potential effects on metabolism and performance. It is known and accepted that ingestion of solid foods significantly slows gastric emptying, digestion, and absorption rates compared with liquid foods [29]. This will have an impact on blood glucose concentration, and because this can be a concern for athletes, it has been suggested that consuming solid food may provide a slower release of glucose to the blood for maintenance of levels during exercise [30]. Interestingly, data from studies comparing ingestion of solid versus liquid CHO [31] and solid versus gel CHO [32] found no significant differences in blood glucose concentrations between groups. Furthermore, additional research has found no differences in CHO oxidation rates between solid versus liquid [33] or liquid versus gel [34] CHO consumed during exercise. Performance studies have found no significant differences following preexercise ingestion of solid versus liquid [32] or solid versus gel [31,32] CHOs. It appears that the form of CHO consumed does not play a significant role in performance or metabolism, and it is therefore suggested that whatever the athlete's preference is to consume can be supported.

Finally, the amount of CHO consumed prior to exercise does not appear to play a major role in performance or changes in blood glucose concentrations. This is particularly true of the emerging area of research examining CHO mouth rinse studies [35–39]. Performance benefits can be seen with these rinses for exercise lasting between 30 minutes to 1 hour, however anything more prolonged would require ingestion of greater amounts of CHO as suggested above [40]. The mouth rinse studies suggest that the presence of CHO in the mouth activate the taste buds stimulating some cranial nerves (VII, IX, X) resulting in a modulation of motor activity through higher brain functions [41–43].

Ultimately, the concerns that should be addressed by athletes prior to competition should be: (1) to ensure that optimal levels of liver and muscle glycogen are attained to sustain performance and (2) if the individual

is concerned that he or she might be a hypoglycemic responder during exercise, to consume low GI foods prior to exercise, consume CHO right before exercise, or avoid CHO completely for at least 60 minutes prior to exercise bouts.

2 CHO Feedings During Exercise and Postexercise

Numerous studies have documented that consumption of CHOs (glucose or glucose polymers) during exercise improves endurance performance in events lasting 60 minutes or longer [31,44,45]. Feeding for bouts lasting less than 60 minutes has produced mixed results [46,47] unless the intensity is high, in which case it appears to be beneficial [48]. The mechanisms appear to be the maintenance of blood glucose levels and the additional CHO to maintain muscular contraction. In the literature, most of the results that support these notions have been obtained using tracer techniques and naturally enriched ^{13}C glucose. What does this body of literature tell us? It suggests that CHO is being oxidized at a rate of approximately 1−1.1 g/minute. This will be influenced by several factors, including: (1) The rate of gastric emptying, which is known to decrease as the concentration of CHO increases and the rate of administration increases; (2) the decreases in absorption that are seen with exogenous levels of CHO administration of 1.2−1.7 g/minute; and (3) the liver, which can potentially release up to 1 g/minute of exogenous CHO to the bloodstream. Due to these factors it is ideal to consume CHOs that utilize multiple transporters. For example, glucose and galactose are absorbed by secondary transport coupled to sodium transport into the epithelial cells and then move in to the blood by facilitated diffusion. Fructose is absorbed through the epithelial cells and into the blood by facilitated diffusion. As a result the glucose can help to maintain the blood sugar levels and the fructose can be moved to the liver to aid in homeostasis. This strategy is particularly important for long duration events (greater than 2.5 hours), in which it is recommended to only consume CHO that will utilize these multiple transporter mechanisms.

CHO restoration postexercise is imperative for athletes, especially for those who train multiple times in one day or on successive days so that performance during these sessions can be at the highest level. Optimizing glycogenesis, the making of glycogen for storage, can be achieved by consuming 7−12 g/kg per body weight per day [1]. This is equivalent to roughly 65% CHO diet when a 70-kg person is consuming 3000 calories (Fig. 13.2). Glycogen synthase (GS-1) is the enzyme responsible for reforming glycogen, and it is aided by GLUT-4 (mentioned previously). When muscle is inactive, GLUT-4 remains in the center of the muscle cell; however, when we are exercising, it is translocated to the cell membrane, allowing glucose to enter the cell, and this starts glycogenesis. Upon cessation of exercise, GLUT-4 does not immediately return to the center of the cell. It is by this premise that high GI CHO foods and drinks may be more favorable for glycogen storage than some low GI food choices [49] because glycogen storage may occur at a slightly faster rate during the first few hours after exercise [12]. Again, this is especially true for athletes who have limited time between workouts. For athletes who have extended time periods of inactivity, it is not as critical to consume CHO immediately; however, these athletes still need to meet the goals for total CHO intake throughout the day.

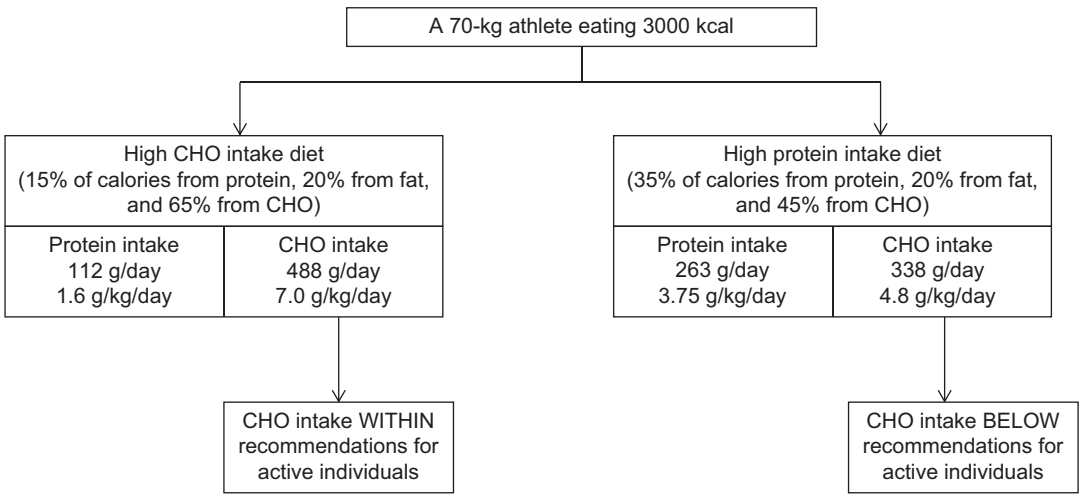

FIGURE 13.2 CHO consumption and meeting daily needs.

In summary, the recommendations for CHOs for after exercise are:

1. Feed approximately 1−1.2 g/kg/hour during the first 4−6 hours of recovery.
2. Over a 24-hour period, feed 7−9 g/kg/body weight for females and 8−12 g/kg/body weight for males.
3. High GI foods provide the best glycogen replacement to increase the insulin response and glucose transport into the cell for glycogenesis.
4. Beverages containing 70−90 g CHO (4−6% CHO) can be used immediately postexercise and consumed at a rate of 1.2 g CHO/kg/hour.

3 CHO Loading (Supercompensation)

CHO loading is a special practice that aims to maximize or "supercompensate" muscle glycogen stores up to twice the normal resting level (e.g., ~500−900 mmol/kg dry weight). The first protocol was devised in the late 1960s by Scandinavian exercise physiologists who found, using the muscle biopsy technique, that the size of preexercise muscle glycogen stores affected submaximal exercise capacity [50−52]. Several days of a low-CHO diet resulted in depleted muscle glycogen stores and reduced endurance capacity compared with a mixed diet. However, high CHO intake for several days caused a "supercompensation" of muscle glycogen stores and prolonged the cycling time to exhaustion. These pioneering studies produced the "classical" 7-day model of CHO loading. This model consists of a 3- or 4-day "depletion" phase of hard training and low CHO intake, followed by a 3- or 4-day "loading" phase of high CHO intake and exercise taper (i.e., decreased amounts of training) [53]. Early field studies of prolonged running events showed that CHO loading enhanced performance not only by allowing the athlete to run faster but also, rather, by prolonging the time that the athlete could maintain the race pace.

Further studies undertaken on trained subjects have produced a "modified" CHO loading strategy [54]. The muscle of well-trained athletes has been found to be able to supercompensate its glycogen stores without a prior depletion or "glycogen stripping" phase. For well-trained athletes at least, CHO loading may be seen as an extension of "fueling up"—involving rest/taper and high CHO intake over 3 or 4 days. The modified CHO loading protocol offers a more practical strategy for competition preparation by avoiding the fatigue and complexity of the extreme diet and training protocols associated with the previous depletion phase. Typically, CHO loading postpones fatigue and extends the duration of steady-state exercise by approximately 20% and improves performance over a set distance or workload by 2% or 3% [55,56]. In summary the recommendation works out to be approximately 10−12 g/kg body weight for every 24 hours prior to competition. While the depletion-taper outlined method above appears to be effective, the 10−12 g/kg body weight over 24 hours needs to be initiated by minimally 36 hours prior to competition to obtain performance benefits [1].

B Fat

Fat represents the primary substrate for aerobic exercise; however; athletes typically avoid fat as a part of their diet. As mentioned previously, this is especially true for athletes for whom body weight and/or composition is an important part of the sport. It would be remiss not to discuss, even briefly, fat and its importance in energy metabolism during exercise because it is the primary substrate used at rest and during low- and moderate-intensity exercise (Fig. 13.3). In addition, exercise training substantially enhances fat oxidation and utilization (Fig. 13.4) by increasing enzyme concentration in the major metabolic pathways associated with fat oxidation; muscles also become more densely populated with capillaries to facilitate greater blood flow and oxygen extraction, and mitochondrial volume is increased (major site for fat oxidative pathways).

Research in this area has focused on gender differences, which seem to support women having a greater

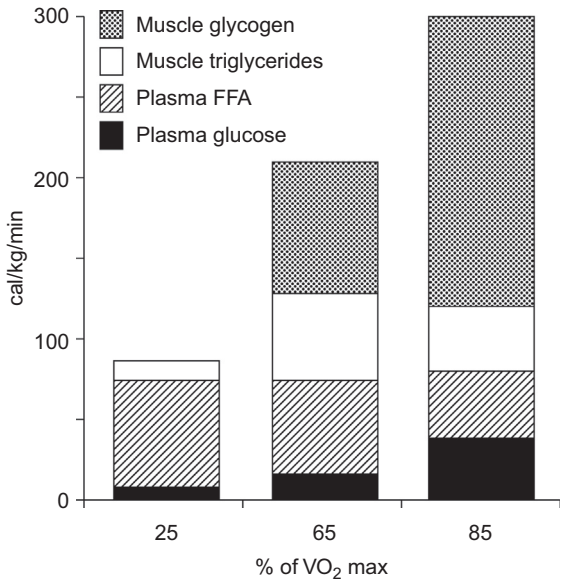

FIGURE 13.3 Contribution of the four major substrates during low (25%), moderate (65%), and high (85%) intensity exercise. During low and moderate intensity, IMTG and plasma-free fatty acids provide the substrate for ATP production, whereas at high intensity muscle glycogen is the major contributor. Reproduced with permission from J.A. Romijn, E.F. Coyle, L.S. Sidossis, A. Gastaldelli, J.F. Horowitz, E. Endert, R.R. Wolf, Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration, Am. J. Physiol. 265 (1993) E380−E391.

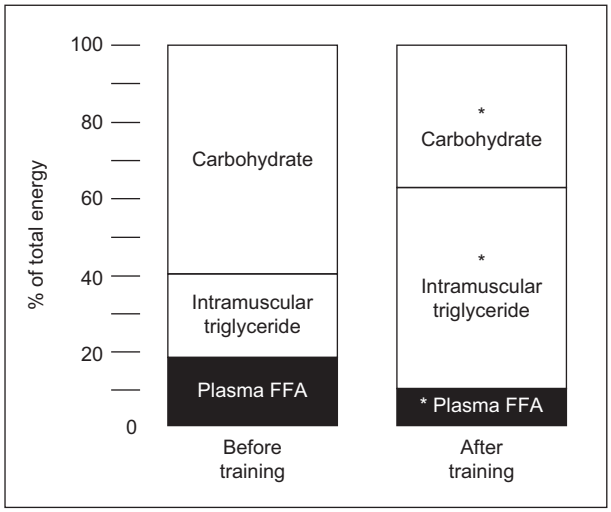

FIGURE 13.4 Changes in substrate use after training. The asterisk denotes a significant increase in using IMTG for ATP production with a significant decrease in CHO use. Reproduced with permission from B.F. Hurley, P.M. Nemeth, W.H. Martin, 3rd, J.M. Hagberg, G.P. Dalsky, J.O. Holloszy, Muscle triglyceride utilization during exercise: effect of training, J. Appl. Physiol. 60 (1986) 562–567.

reliance on fat oxidation compared to men. Several studies specifically showed that women increased the amount of intramuscular triglycerides (IMTG) stored in the muscle and had a significant decrease in IMTG postexercise compared to men [33,34,57]. These men and women were matched for VO_2 mL/kg FFM/minute and training volume, and the women were tested during the same menstrual phase to control for the influence of sex hormones. These studies suggest that athletes, especially female athletes, should consume enough fat in the diet to adequately replenish what they use during their training sessions. It has been shown that women who consume low-fat diets and who perform endurance events can have depleted IMTG levels 2 days after these events. This will compromise performance [58–61], and as such it is recommended that for females who perform this type of exercise, energy intake from fat can be as high as 30% of daily caloric intake. Furthermore, low-fat diets also compromise the intake of the important fat-soluble vitamins A, D, E, and K as well as the essential fatty acids (α-linolenic acid and linoleic acid) [62].

There are no specific recommendations for fat intake for athletes as there are for CHO and protein (discussed next) other than not to restrict levels that would be detrimental to health by limiting important vitamin and essential fatty acid intake. It is important to remind the athlete that fat oxidation is the primary way to produce ATP during aerobic exercise, and IMTG stores need to be replaced like the glycogen stores. Athletes should be advised to take in healthy fats such as mono- and polyunsaturated fatty acids. These are healthier and examples include consuming foods such as nuts, using olive oil for cooking, and consuming "fatty" fish, which contain the essential fatty acids.

1 Fat Adaptation

Interest in metabolic retooling for the optimization of fat utilization among well-trained endurance athletes has gained significant headway due to its theoretical applicability. Of note, metabolic adaptations that enhance the ability of skeletal muscle to use fat as a substrate during exercise may be advantageous in that an increase in fat utilization promotes a glycogen sparing effect. In theory, these metabolic adaptations have the potential to improve performance and delay fatigue by allowing the athlete to take full advantage of the almost unlimited capacity of fat to generate energy during continuous exercise. In practice however, there appears to be no performance advantage with fat adaptation despite distinct changes in substrate metabolism.

The traditional fat adaptation protocol involves the consumption of a low-CHO, high-fat diet (LCHF) for approximately 14 days while maintaining current training volume and intensity, whereby roughly >60% of energy is derived from fat and <25% from CHO [63,64]. The goal of this dietary intervention is to maximize IMTG stores, increase rates of whole-body and muscle fat oxidation, and reduce rates of muscle glycogen breakdown (glycogenolysis) specifically during submaximal exercise [63]. Alternatively, research suggests that this period for fat adaptation is far shorter than what is traditionally practiced as significant increases in fat oxidation and decreases in CHO oxidation can be achieved within only 5–10 days [65]. As such, significant metabolic adaptations can surely be achieved under CHO-restricted dietary conditions.

One of the key mechanisms by which a LCHF diet suppresses CHO oxidation is by regulating the activity of pyruvate dehydrogenase (PDH), the mitochondrial enzyme responsible for converting pyruvate to acetyl-CoA. This is accomplished primarily through the activation of PDH kinase (PDK), an enzyme that inactivates PDH through phosphorylation. In short, a rise in acetyl-CoA as a result of CHO restriction significantly upregulates the activity of PDK which moves PDH into a less active form, shifting fuel reliance away from CHO and more toward fat [66–68]. In addition, chronic intake of LCHF diets can lead to dramatic increases in fat oxidation rates and a greater reliance on fat metabolism at higher exercise intensities. Of note, a recent study showed that ultra-endurance runners who habitually consume (for an average of 20 months) a LCHF (10% CHO; 19% PRO; 70% FAT) have a 2.3-fold higher peak fat oxidation rate

(1.54 g/minute vs 0.67 g/minute) when compared to those who consume a traditional high-CHO diet (59% CHO; 14% PRO; 25% FAT) [69]. Further, peak fat oxidation rates were achieved at higher exercise intensities in athletes who consumed a LCHF diet (70% VO_2 max vs 55% VO_2 max) and also demonstrated a significantly greater energy contribution from fat during submaximal exercise intensities (88% vs 56%). These results further corroborate the efficacy of fat adaptation in improving fat utilization during prolonged exercise. It is important to note, however, that both short-term fat adaptation (1−3 days) and complete fat adaptation (2 weeks) can result in a reduction of resting glycogen stores and therefore cannot be considered "true glycogen sparing" despite changes in CHO oxidation rates [70,71]. Thus, a key strategy to restore precompetition glycogen levels and maintain elevated fat oxidation rates is to implement a dietary periodization protocol that includes a CHO restoration period (1−3 days) after fat adaptation is achieved; the details of which are outlined extensively here [63]. Interestingly, research shows that CHO restoration can be accomplished even sooner, within a single day [72−74].

Despite extensive metabolic retooling as a result of LCHF diets, there still appears to be no performance advantage at submaximal intensities and current research lacks sufficient evidence to suggest otherwise (for a more recent review, see Ref. [64]). In contrast, fat adaptation can compromise performance at higher intensities as demonstrated by Havemann et al. [75]. Here, they showed that 100-km time trial performance was not significantly different in endurance-trained male cyclists when fed either a high-fat diet (HFD) or high-CHO diet with one day of CHO loading (∼90% energy from CHO) after 6 days of fat adaptation. Mean power output was significantly reduced during high-intensity 1-km sprints in the HFD group throughout the 100-km time trial however. These findings highlight an important observation in that although fat adaptation confers no performance advantage at submaximal intensities, submaximal exercise capability postfat adaptation is comparable to that of higher CHO diets as reported in earlier studies [76,77]. Furthermore, endurance events such as the one simulated in Havemann's study may contain intermittent sprint intervals that require exercise intensities approaching near maximal effort. In this scenario, fat adaptation can restrict performance by limiting anaerobic exercise capacity.

In summary, fat adaptation results in a significant metabolic retooling that optimizes fat oxidation and reduces fuel reliance on CHO at submaximal intensities. Further, submaximal performance postfat adaptation is significantly comparable to performance based on a higher CHO diet. Although fat adaptation can result in a reduction of resting muscle glycogen concentrations, such detriments can be attenuated with a short CHO loading period (1−3 days) prior to competition day. However, fat adaptation can significantly limit performance at maximal intensities and the physiological demands of a given endurance event must therefore be strongly considered when determining appropriate dietary interventions. For the athlete, personal preference is equally important to consider as motivation and drive are profound determinants of sport performance. As such, implementing dietary strategies that they feel best facilitates their performance, and lifestyle is essential.

C Protein

There is still considerable debate about how much dietary protein is required for optimal athletic performance. This is most likely because muscle contains a large proportion of the total protein in a human body (∼40%). Muscle also accounts for 30−50% of all protein turnover in the body. In muscle, the majority of amino acids are incorporated into tissue proteins, with a small pool of free amino acids. This pool undergoes turnover, receiving free amino acids from the breakdown of protein and contributing amino acids for protein synthesis. Protein break in skeletal muscle serves two main purposes: (1) to provide essential amino acids when individual amino acids are converted to acetyl CoA or tricarboxylic acid cycle intermediates. (2) In addition it provides individual amino acids that can be used elsewhere in the body for the synthesis of neurotransmitters, hormones, glucose, and proteins. If protein degradation rates are greater than the rates of synthesis, there will be a reduction of protein content; conversely, muscle protein content can only increase if the rate of synthesis exceeds that of degradation.

Exercise (especially endurance exercise) results in increased oxidation of the branched-chain amino acids, which are essential amino acids, and cannot be synthesized within the body. Therefore, increased oxidation would imply that the dietary protein requirements are increased. Some studies in which the nitrogen balance technique was used showed that the dietary protein requirements for athletes involved in prolonged endurance training were higher than those for sedentary individuals. Whether requirements are really higher remains somewhat controversial (for review, see Refs. [78,79]).

It has been estimated that protein may contribute up to approximately 15% to energy expenditure in resting conditions. During exercise, this relative contribution is likely to decrease because energy expenditure is increased and most of this energy is provided by CHO and fat. During very prolonged exercise when CHO availability becomes limited, the contribution of protein to energy expenditure may amount to approximately 5% of the total energy expenditure. Thus, although protein oxidation is increased during endurance exercise, the relative contribution of protein to energy expenditure remains small. Protein

requirements may increase somewhat, but this increased need may be met easily by a moderate protein intake. The research groups that advocate an increased protein intake for endurance athletes usually recommend a daily intake of 1.2−2.0 g/kg body mass. This is approximately twice the level of protein intake that is recommended for sedentary populations.

There are reports of increased protein breakdown after resistance exercise. The suggested increased dietary protein requirements with resistance training are related to increased muscle bulk (hypertrophy) rather than increased oxidation of amino acids. Muscle protein breakdown increases after resistance training, but to a smaller degree than muscle protein synthesis. The elevations in protein degradation and synthesis are transient. Protein breakdown and synthesis after exercise are elevated at 3 and 24 hours after exercise but return to baseline levels after 48 hours. These results seem to apply to resistance exercise and high-intensity dynamic exercise.

There is controversy regarding whether strength athletes really need to eat large amounts of protein. Nitrogen balance studies conducted on such athletes have been criticized because they generally have been of short duration and a steady-state situation may not be established [79]. The recommendation for protein intakes for strength athletes is therefore generally 1.4−2.0 g/kg, depending on body mass, per day. Again, this seems to be met easily with a normal diet, and no extra attention to protein intake is needed. Protein supplements are often used but are not necessary to meet the recommended protein intake. There is also no evidence that supplements would be more effective than normal foods. An exception to the rule is athletes who need to drop weight in order to compete in a weight-dependent sport like boxing or martial arts, as such, exceeding the current recommendation may be advantageous. These athletes are often required to implement short periods of hypoenergetic weight loss for many reasons such as competing in a certain weight class, to improve aesthetic appearance or to improve physical performance. In general, rapid weight loss is usually achieved through fluid restriction (dehydration) and increased exercise, while gradual weight loss is commonly achieved through calorie restriction (−75 to 130 kJ/kg/day for >1 week) [80]. As such, loss of lean body mass may compromise performance and an increased protein intake may offset these detriments when intense training is continued [78,80]. Specifically, research suggests that a protein intake of ∼2.3 g/kg (or ∼35% protein in diet) significantly attenuates lean body mass loss in athletes during these periods of hypoenergetic weight loss [81].

In conclusion, it is very important to understand the difference between complete and incomplete proteins when planning meals. Complete proteins contain all of the essential amino acids (those that the body cannot produce), including the branch-chain amino acids. Food sources include meats, fish, poultry, eggs, and dairy. Incomplete proteins are those that do not contain all of the essential amino acids and are typically plant-based sources such as legumes and grains. Animal protein sources (complete proteins) have a high digestibility, whereas the plant sources do not. This is not to suggest that athletes cannot be vegetarian; however, it does suggest that these athletes need to pay particular attention to their protein intake and ensure that they are eating complimentary proteins. This means they must eat a variety of plant-based protein sources to ensure that they get all of the essential amino acids required each day.

IV MICRONUTRIENT REQUIREMENTS FOR ATHLETES

A Vitamins

Vitamins are essential organic compounds that serve to regulate metabolic processes, energy synthesis, and neurological processes and to prevent cell degradation and/or death. Vitamins can be either water or fat soluble. Fat-soluble vitamins are A, D, E, and K (as previously mentioned), and examples of water-soluble vitamins are C and the B vitamins. The body can store fat-soluble vitamins and therefore consumption of these in excess can result in toxicity. In contrast, water-soluble vitamins cannot be stored by the body, with a few exceptions such as vitamin B_6, so they are excreted in the urine when consumed in excess. Research has suggested that some vitamins may possess some health benefits (e.g., vitamins C and E, niacin, and folic acid); few of these have been shown to enhance performance in athletes when sufficient intake is present.

Studies have reported that active individuals tend to be deficient in certain B vitamins including B_6, thiamin, and riboflavin [82,83]. The B vitamins are an important group because they act as coenzymes in metabolism. This means that they can help donate or accept methyl group items, especially in substrate metabolism. For example, the active form of thiamin, thiamin pyrophosphate, can help to remove carbon dioxide and hydrogen ions from pyruvate to help create acetyl CoA. Acetyl CoA is the molecule required to enter the Kreb's cycle and initiate oxidative metabolism. As a result, deficiency in the B vitamins can be detrimental to energy metabolism. In addition, vitamin B_{12} and folate deficiencies manifest themselves as either pernicious or megaloblastic anemia, respectively. This is a serious concern as well because improper red blood cell function will adversely affect the oxygen carrying ability of the body, which will limit performance. Finally, for vegetarian athletes, vitamin B_{12}

should be carefully monitored because the best sources of B vitamins are found in meat products; as such, supplementation of B_{12} in vegetarian athletes is essential.

Other vitamins, such as A, C, and E, are known antioxidants. This means they have the ability to scavenge free radicals and decrease oxidative stress. This may be of particular interest to athletes because theoretically it may help athletes to tolerate heavy training loads and potentially accelerate recovery. However, the literature on antioxidant supplementation is convoluted and in some cases inconclusive. What is known is that athletes do have enhanced antioxidant status and enzymes as a result of exercise training [84]. In addition, it has been suggested that supplying antioxidants to athletes may actually negate the natural antioxidant health-promoting effects of exercise [85]. As such, caution should be exercised with regard to the efficacy on intake and the purpose for supplementation.

Finally, vitamin D is important (Recommended Daily Allowance (RDA) 600 IU/day for males and females aged 19−50 years). Its partnership with calcium and their role in bone health are well-established [86]. Although this vitamin is typically not a concern with regard to deficiency, it can be in sports in which body composition is an issue. This has previously been mentioned and it is likely because these athletes tend to underconsume fat, which will be reflected in the status of the fat-soluble vitamins and essential fatty acid intake. As a result, it is recommended that these types of athletes would benefit from supplementation of vitamin D.

In summary, athletes who consume a balanced diet with appropriate calorie intake will meet their vitamin requirements and there is no need for additional supplementation. However, as mentioned previously, athletes do tend to have some dietary inadequacies and as such a multivitamin is sometimes recommended to ensure that all vitamin requirements are met.

B Minerals

Minerals are essential inorganic compounds that help with tissue structure and metabolic processes such as components of enzymes and hormones and regulators of metabolic and neural control. As is the case with vitamins, some minerals have been found to be deficient in athletes—e.g., iron (RDA 8 mg/day for males and 18 mg/day for females aged 19−50 years) and calcium (RDA 1000 mg/day for males and females aged 19−50 years). Lacking these minerals will negatively impact performance. Conversely, supplementing these minerals can improve performance.

Exercise is an outstanding form of weight-bearing activity; however, many athletes still have low bone density, especially female distance runners. The more common problem with this group of athletes, however, is menstrual disturbances that disrupt hormonal status and predispose women to the female athlete triad (disordered eating, low bone density, and menstrual dysfunction). In these athletes, calcium supplementation may be increased to 1200−1500 mg/day, and this has been shown to help maintain bone mass.

Iron is a common mineral found to be deficient especially in early training periods. This is reflected by reductions in both hematocrit (percentage of red blood cells) and hemoglobin (oxygen-carrying component of the red blood cell). This can be due to several factors, including changes in plasma volume that dilute hemoglobin, exertion hemolysis, and dietary practices that include low iron intake, low iron bioavailability, and increased iron excretion. Heme iron, such as that found in meats, fish, and poultry, is better absorbed and bioavailable compared to nonheme iron, which is found in grains, vegetables, and legumes. Factors that enhance iron absorption include vitamin C, peptides from fish/meat/chicken, alcohol, and food acids, whereas factors that inhibit absorption include phytates, polyphenols, calcium, and peptides from plant sources such as soy protein. The absorption of both heme and nonheme iron is increased as an adaptive response in people who are iron deficient or have increased iron requirements. Prevention and treatment of iron deficiency may include iron supplementation, with a recommended therapeutic dose of 100 mg/day of elemental iron for 2 or 3 months. However, the management plan should include dietary counseling to increase the intake of bioavailable iron and appropriate strategies to reduce any unwarranted iron loss.

In addition, zinc and magnesium are two other minerals that are important for energy production. Zinc plays a role in growth, building, and repair of muscle tissue; energy production; and immune function. Survey data suggest that Americans have zinc levels below the recommended intake [87−89] and that female athletes are at a particular risk for deficiency [89]. Zinc levels can be difficult to measure; however, deficiency has serious consequences, including decreases in cardiorespiratory function, muscle strength, and endurance. Vegetarian athletes are again at a particular risk for zinc deficiency. The RDA for zinc is currently 11 mg/day for males and 8 mg/day for females. There is no reason to consume more than 40 mg/day because this is the upper limit for toxicity [87]; however, intake at 25 mg/day has been shown to minimize exercise-induced changes in immune function [90−93]. Magnesium plays a role in cellular metabolism; membrane stability; and neuromuscular, immune, and hormonal status. Magnesium deficiency impairs performance due to inefficient oxygen use, and in sports in which weight is an issue; deficiency has been reported [94,95]. The RDA for magnesium is 420 mg/day for males and 320 mg/day for females.

V FLUID REQUIREMENTS FOR ATHLETES

Water represents approximately 50–60% of total body weight and functions as a transport medium, lubricant, solvent, and thermal regulator (Fig. 13.5). The body is constantly exchanging fluids between the interstitial fluid (fluid between tissues) and intracellular fluid (fluid within the cells) compartments to maintain both hydrostatic and osmotic pressures. The maintenance of these fluid compartments occurs through osmotic forces that are regulated by properly working sodium–potassium pumps. Sodium controls the extracellular fluid (fluid outside the cell) volume, regulates osmolality, helps with acid–base balance, and maintains resting cell membrane potential. Potassium controls the intracellular fluid volume, and it also regulates osmolality, acid–base balance, and cell membrane potential. The body has active mechanisms in place for monitoring sodium levels, ensuring that they are always in acceptable ranges. No such mechanism exists for potassium; potassium simply relies on ion exchange pumps for maintenance.

When we sweat during exercise, we lose fluid from the interstitial fluid compartment and this will disrupt balance, calling on our electrolytes to help correct the disruption. The issue is that when we sweat, we also lose electrolytes, so the replenishing of the fluid and electrolytes before, during, and after exercise sessions is of utmost importance. Regulation of fluid balance is dictated by our thirst mechanism (the sensation of feeling thirsty is not perceived until a person has lost at least 2% of body mass), monitoring of the extracellular fluid volume, and blood pressure. To maintain fluid balance, water intake may vary from 1 L to approximately 12 L/day. However, during exercise and especially during exercise in hot conditions, sweat rates (and thus water losses) may increase dramatically and dehydration may occur (i.e., the body is in negative fluid balance). Depending on the sport, sweat rates can range from 0.3 to 2.4 L/hour [96], with the average sodium concentration in sweat approximately 1 g/L [97].

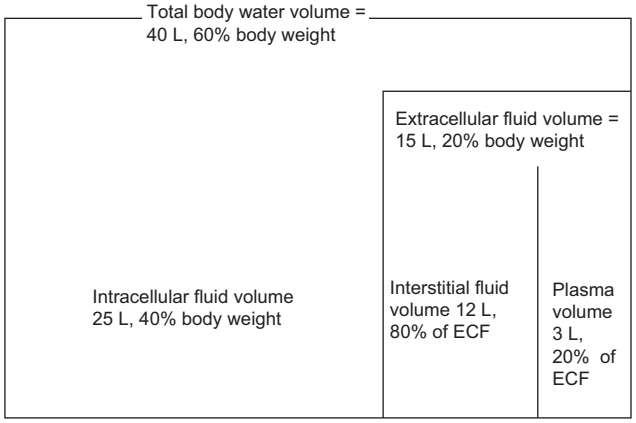

FIGURE 13.5 Body water distribution.

A Dehydration and Performance

The literature is clear that dehydration will negatively impact performance (Fig. 13.6). Dehydration can cause several physiological disturbances, all of which negatively impact performance (Fig. 13.7). Several studies have shown that mild dehydration, equivalent to the loss of only 2% body weight, is sufficient to significantly impair exercise performance [96,98]. In addition, it is often reported that greater losses result in greater reductions in performance. Even very low-intensity exercise (i.e., walking) is affected by dehydration. The capacity to perform high-intensity exercise that results in exhaustion within only a few minutes has been shown to be reduced by as much as 45% by prior dehydration (2.5% of body weight) [99]. Although there is little opportunity for sweat loss during such short-duration, high-intensity events, athletes who travel to compete in hot climates are likely to experience acute dehydration, which can persist for several days and can be of sufficient magnitude to have a detrimental effect on performance in competition. Although dehydration has detrimental effects especially on performance in hot conditions, such effects can also be observed in cool conditions. Both decreases in maximal aerobic power (VO_2 max) and decreases in endurance capacity have been reported with dehydration in temperate conditions [100], although not all studies have found such an effect [101,102].

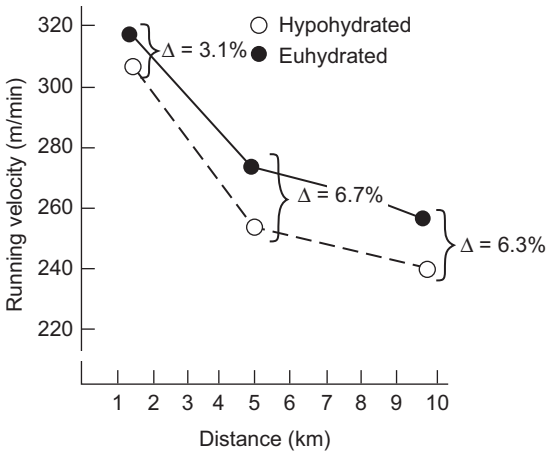

FIGURE 13.6 Effects of dehydration on running velocity over distance covered. Results clearly show that velocity is significantly different at all three distances. Reproduced with permission from M.N. Sawka, R.P. Francesconi, A.J. Young, K.B. Pandolf, Influence of hydration level and body fluids on exercise performance in the heat, J. Am. Med. Assoc. 252 (1984) 1165–1169.

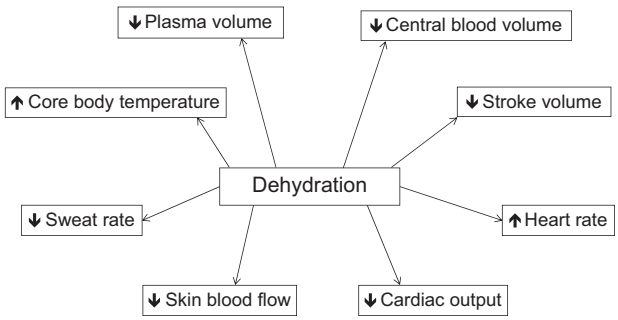

FIGURE 13.7 Physiological side effects of dehydration.

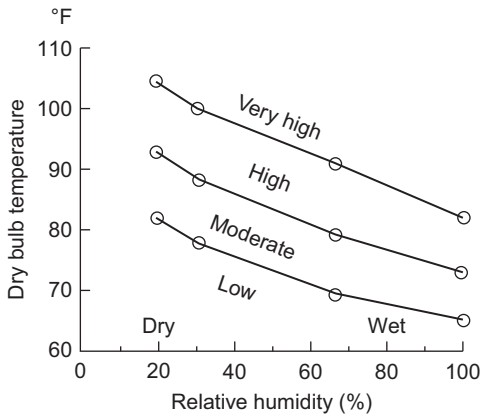

FIGURE 13.8 Temperature, relative humidity, and risk for heat illness. Reproduced with permission from L.E. Armstrong, C.M. Maresh, Fluid replacement during exercise and recovery from exercise, in: E.R. Burkirk, S.M. Puhl (Eds.), Body Fluid Balance: Exercise and Sport, CRC Press, Boca Raton, FL, 1996, pp. 259–281.

B Dehydration and Heat Illness

Dehydration also puts the athlete at increased risk for heat illness. Heat illness can be assessed by calculating wet bulb globe temperature (WBGT) index = 0.7 T_{wb} + 0.2 T_g + 0.1 T_a, where T_{wb} is the wet-bulb temperature, T_g is the globe temperature, and T_a is the ambient or dry bulb temperature. Risks based on this formula are as follows: very high risk; WBGT > 28°C (82°F)—race should be postponed, rescheduled, or canceled. High risk; WBGT 23–28°C (73–82°F)—runners should be aware that heat illnesses may occur, particularly in susceptible persons. Moderate risk; WBGT 18–23°C (65–73°F)—remind runners that heat and humidity will increase during the race if early in the day. Low risk; WBGT < 18°C (<65°F)—does not guarantee heat illness will not occur (Fig. 13.8). Early symptoms of heat injury are excessive sweating, headache, nausea, dizziness, a reduced consciousness, and impaired mental function. When core body temperature rises to more than 40°C, heat stroke may develop; heat stroke is characterized by hot dry skin, confusion, and loss of consciousness.

C Hyponatremia

Hyponatremia or low sodium level is defined by dilution of serum sodium from normal levels of 135–145 to less than 130 mEq/L. Causes of hyponatremia include sodium loss, water retention, or both due to excessive sweating, vomiting, diarrhea, or excessive use of diuretics. The most common symptoms, if water intake is excessive and hyponatremia develops slowly, are mental confusion, giddiness, and coma. Muscle twitching, irritability, and convulsions occur if the development is rapid.

Hyponatremia appears to be most common among slow runners in marathon and ultramarathon races and probably arises because of the loss of sodium in sweat coupled with very high intakes of water. This means that there can be a danger of misdiagnosis of this condition when it occurs in individuals participating in endurance races. The usual treatment for dehydration is administration of fluid intravenously and orally. If this treatment were to be given to a hyponatremic individual, the consequences could be fatal.

D Fluid Intake Before, During, and After Exercise

The American College of Sports Medicine's (ACSM) position stand [96] on exercise and fluid replacement provides a comprehensive review of the research and recommendations for hydration before, during, and after exercise. ACSM has also published position stands on exercising in environmental conditions [103,104]. This section summarizes the position stand on fluid replacement and serves as a guide that athletes should follow.

1 Before Exercise

Athletes should start drinking at least 4 hours prior to exercise approximately 5–7 mL/kg body weight of water or sport beverage. This allows for adequate time to optimize hydration status and excrete any excess fluids in the urine prior to competition when it would not be timely to do so. Over-hydrating (hyperhydration) with excess water or glycerol solutions will substantially expand intra- and extracellular fluid volumes and will increase the risk of voiding during the competition. In addition, it confers no clear performance benefit.

2 During Exercise

As mentioned previously, decreases that are 2% or more of body weight initiate the thirst response and are typically indicative of dehydration, which is detrimental to performance. Fluid intake during exercise should be aimed at preventing this from occurring. Although this seems easy in theory, this is highly dependent on the

athlete due to body size, sweat rate, and the exercise being performed. In addition, fluid balance during exercise may not occur because sweat rates may be greater than gastric emptying rates that limit fluid absorption and ultimately fluid ingestion rate by the athlete. To help, gastric emptying rates can be maximized when the stomach is full, and absorption rates can be maximized when the CHO concentration in the beverage is approximately 6−8%. Athletes should start drinking fluids right away during exercise and at regular intervals to replace water loss. Athletes should ingest fluids cooler than the ambient temperature (15−22°C). CHO−electrolyte drinks are beneficial for exercises that last longer than 1 hour; these help replace electrolytes and maintain blood glucose levels. The sodium level in fluids when exercise lasts longer than 1 hour should be approximately 0.5−0.7 g/L.

3 After Exercise

Because most athletes will not consume enough liquids during exercise, postexercise hydration is critical. If the athlete has adequate time between exercise sessions, then intake of normal meals and beverage should replenish hydration status. Rapid and complete recovery from exercise can be accomplished by drinking at least 16−24 oz (450−675 mL) of fluid for every pound of body weight lost during the exercise bout. This can easily be tracked by simply weighing oneself before and after exercise and then hydrate accordingly. Another easy way to determine hydration status is to observe the color of the urine (Fig. 13.9).

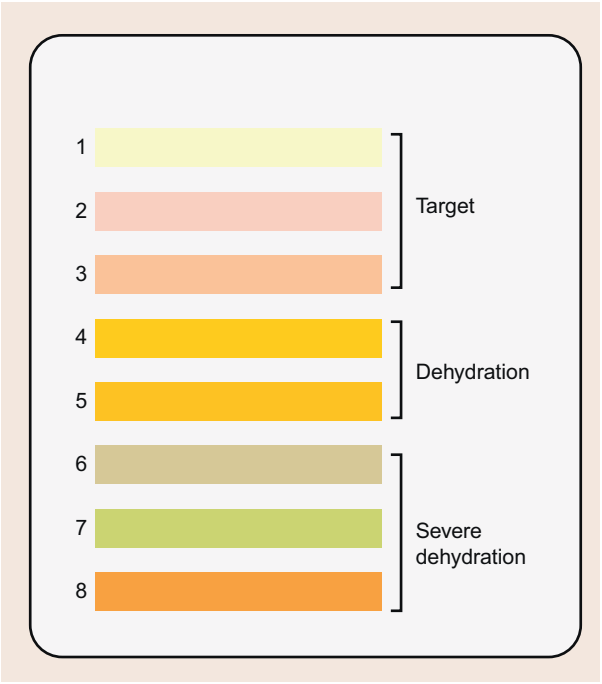

FIGURE 13.9 Urine color hydration chart. Scientific validation for this chart can be found in the *International Journal of Sport Nutrition*, vol. 4 (1994) 265−279 and vol. 8 (1998) 345−355.

VI SUMMARY AND CONCLUSIONS

The basic difference between athletes and the general population is that athletes typically need more fluids to recover sweat and electrolyte loss during physical activity and greater food intake to maintain energy balance demands that are associated with exercise. Much of this energy can be in the form of CHO, but neglecting protein or fat could be detrimental to performance. Although there are general rules for how to govern these increased energy demands, each individual is different and the concept of one diet fits all is obsolete. However, the types of foods recommended can be standard in that good CHOs in the forms of grains, fruits, vegetables, and breads should be encouraged along with fats that are high in mono- and polyunsaturated fatty acids such as nuts, olive oil, and "fatty" fish and proteins that are lean and complete (e.g., that contain all of the essential amino acids) such as meat, poultry, and fish. Requirements for these macronutrients may vary between strength-trained and aerobic-trained athletes and also may vary between genders. Special attention is warranted for sports that place a strong emphasis on body composition and weight to ensure that these athletes are consuming enough calories. Furthermore, vegetarian athletes need to be mindful of ensuring that they are consuming complimentary proteins to obtain all of the essential amino acids recommended as well as vitamin B_{12}, which is typically found only in animal products. In addition, female athletes, especially in body composition and weight-dependent sports, need to be counseled on the importance of maintaining adequate calorie intake for both macro- and micronutrients to avoid disturbances such as those seen in the female athlete triad. Consuming adequate nutrients is essential for an athlete to achieve optimal performance. The guidelines put forth in this chapter provide a thorough overview of the evidence-based nutritional recommendations to help athletes understand energy requirements allowing for both energy balance and peak performance.

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Chapter 14

Nutrition for Children With Special Health Care Needs

Beth Ogata[1], Hope Wills[2] and Marion Taylor Baer[3]

[1]University of Washington, Seattle, WA, United States, [2]University of Southern California, Los Angeles, CA, United States, [3]University of California at Los Angeles, Los Angeles, CA, United States

I INTRODUCTION

According to data available from the National Survey of Children with Special Health Care Needs, 11.2 million children in the United States (15.1%) have special conditions that increase their need for health care services and educational supports beyond those of children without special needs [1]. Children with special health care needs include those with specific diagnoses related to developmental disabilities such as genetic disorders (e.g., trisomy 21 (Down syndrome) and phenylketonuria (PKU), cognitive and behavioral challenges (e.g., autism and intellectual disabilities), and neuromotor impairments (e.g., cerebral palsy and spina bifida). Children who are born prematurely or with a low birth weight are considered to be at high risk of developing a special health care need. There are also children with common chronic health concerns (e.g., asthma, diabetes, and obesity) who can be included in the broader definition of children with special health care needs, but the focus of this chapter is on children with functional conditions related to prematurity or a known developmental disability.

As many as 40−60% of children with special health care needs are at risk of one or more problems with nutritional status, ranging from slow growth and poor feeding to more severe gastrointestinal problems and metabolic disorders [2,3]. Specific segments of the population experience greater impact. Brooks et al. [4] illustrated the impact of weight status on morbidity and mortality in children with cerebral palsy. Children with the highest degree of neuromotor dysfunction (Gross Motor Function Classification System—GMFCS V) and in the lowest quintile for weight-for-age, demonstrated higher rates of morbidity and mortality than children with less severe motor dysfunction. Table 14.1 provides a list of special health care needs, including the sequelae of prematurity, the more common genetic disorders, and other developmental disabilities, and the associated functional problems with nutrition. Because of the increasing prevalence of children with special health care needs, the essential role that nutrition therapy plays in treating several conditions, and the high risk of developing comorbid nutritional problems (e.g., underweight and overweight), nutrition professionals will encounter children with special health care needs in their practices and should be able to anticipate their most commonly seen nutrition problems [5,6].

Intrauterine nutrition insults occurring prenatally can predispose children to developmental disabilities and poor growth, as well as to problems with obesity and chronic illnesses later in life. Children with special health care needs receive services in multiple settings, including prenatal public health nutrition programs such as WIC (the Special Supplemental Nutrition Program for Women, Infants and Children; http://www.fns.usda.gov/wic), early intervention programs, schools, and primary and tertiary care practices. To ensure that those at high risk of nutrition problems are identified and referred to appropriate services, nutrition screening is an important part of care in all of these settings [7,8]. Evidence establishing the cost-effectiveness of appropriate nutrition interventions, especially preventive interventions, for high risk populations of mothers and children is mounting [2,9,10]. Mothers who receive adequate nutrition during pregnancy have better birth outcomes, and children who have their nutritional risks identified and receive appropriate intervention

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TABLE 14.1 A Comparison of Specific Developmental Conditions with Types of Nutrition Risks

	Growth			Diet		Medical		
Disorder	Underweight	Over-weight	Short Stature	Low Energy Needs	High Energy Needs	Feeding Issues/ Special Diet	Constipation	Chronic Medications
Autism	X		X			X		X
Prematurity with Bronchopulmonary Dysplasia	X		X		X	X		X
Cerebral Palsy	X	X	X	X	X	X	X	X
Down Syndrome		X	X	X		X	X	
Inborn Errors of Metabolism						X		X
Prader-Willi Syndrome		X	X	X		X		
Spina Bifida	X	X	X	X		X	X	X

demonstrate better growth, health, and developmental outcomes than those who do not.

This chapter describes nutritional risk for mothers during pregnancy that affects their offspring and for infants and children identified with a special health care need, with the intent to prevent disability through good nutrition, to provide secondary prevention of further disability in specific conditions with known nutrition issues, and to preserve and promote function in order to enable the child with special health care needs to reach his or her potential in terms of growth and development.

Section II addresses primary and secondary prevention of developmental problems by examining the evidence that nutrition—and specific nutrients—during fetal development and infancy affect developmental outcomes and subsequent special needs for children.

Section III outlines the functional approach to identifying nutritional needs—how growth, diet, and medical issues are assessed, including issues specific to children with special health care needs in each of these areas.

Section IV covers selected pediatric conditions in more depth—inborn errors of metabolism, Prader-Willi syndrome, feeding problems, and autism spectrum disorders—including a description of the diagnosis, assessment of nutritional status, and ongoing intervention, where there is evidence for nutrition interventions.

II THE ROLE OF NUTRITION IN PREVENTING DEVELOPMENTAL PROBLEMS

An optimal supply of nutrients, both macro and micro, may be the most important environmental factor in determining health throughout the lifespan and across generations [11]. Low birth weight (LBW), resulting from preterm birth or intrauterine growth restriction, has long been established as a major risk factor for developmental disabilities (discussed next), but, relatively recently, maternal and even paternal nutritional status is being considered to be more important than previously recognized as part of the life course approach to health development, which characterizes health as the result of the accumulation of both positive and negative environmental impacts over a lifetime, rather than simply one's genetic makeup alone. As conceived by Kotelchuck and Fine [12], these impacts are either viewed as occurrences over the lifespan which are mutable (**timeline**), or, if sustained at critical developmental stages, the impact may be irreversible (**timing**). These positive or negative factors are also dependent on environmental determinants which may be beyond the control of the individual, such as living in poverty or being exposed to toxins (**environment**) and, finally, they may be related to the inequities, such as access to health care—a problem faced by large numbers of individuals worldwide (**equity**). A description of the role of nutrition in terms of the life course perspective and these concepts: timing, timeline, equity, and environment (also called T2E2) has been published elsewhere [13], as has evidence for the importance of nutrition to overall life course health development [14]. This evidence includes epidemiological and experimental studies which suggest that preconceptional, and even intergenerational, nutrition plays a key role in the later development of obesity and its associated chronic diseases—the theory that early "nutrition programming" determines the set point of energy balance in the fetus [15].

Preconception and interpartum nutritional care is now clearly understood to be an essential part of comprehensive infant health strategies [16]. The Centers for Disease Control and Prevention (CDC) has recommended that women of reproductive age receive preconception counseling to improve intakes of calcium, folic acid, iodine, iron, and essential fatty acids; [17] also mentioned in an earlier publication were vitamins A and D, with lesser strength of evidence [18]. The following brief review of research findings illustrates the growing awareness of the associations between nutrition and pregnancy outcome in terms of disability, as well as chronic illness.

A Low Birth Weight (LBW) and Preterm Birth

Risk of preterm birth (before 37 weeks of gestation), LBW (<2500 g), or being small for gestational age (SGA) increases by approximately 50% in the United States (US) with short interpregnancy intervals (<18 months) and less than 2 years postmenarche in young adult women compared to those who are older or who have an interpregnancy interval of 18–23 months; this cannot be entirely explained by social or behavioral factors [19]. Evidence is accumulating that if the pregnant woman is still growing herself or has not had sufficient time since her last pregnancy to replace depleted stores, the physiological changes that normally favor improved nutrient utilization during gestation may not be adequate to meet the needs of both mother and fetus. In such cases, it appears that nutrients are partitioned between the mother and the fetus depending on the initial nutritional status of the mother [19,20]. This underscores the importance of optimal nutritional status of women, from the preconception period and throughout pregnancy, detailed in a recent publication by the International Federation of Gynecologists and Obstetricians (FIGO), on optimal pregnancy outcomes and the prevention of low birth weight as well as defects such as neural tube defects (NTDs) [11]. In one study, the incidence of preterm birth was lowered in women given a diet of fish, oils, low-fat meats and dairy, whole grains, fruits, vegetables, and legumes compared to women who continued their usual diet. Although the study goal was to lower cholesterol in the mothers, it is likely that this diet also improved their nutritional status [21], and evidence that this is likely the case has been recently shown in another study where consuming a "DASH" diet (similar to that described above) resulted in reduced odds of preterm births [22]. Other studies have shown that zinc [23], folate [24], and iron [25] deficiencies all increase the risk of preterm birth and LBW. In addition, low serum selenium early in pregnancy has been shown to predict lower birth weight [26].

A study of women in the Danish National Birth Cohort (~36,000 subjects) indicates that periconceptional use of multivitamins, particularly regular use, is associated with a lowered risk of infants being born SGA [27]. This study also showed that the association between pre- and postmultivitamin use and preterm birth varied according to prepregnancy weight; women whose body mass index (BMI) was less than 25 had significantly less risk of either preterm labor or delivery, whereas the same was not true for overweight women [27]. In a report that was part of the "Generation R" study—a large (>6000 women) but prospective study in The Netherlands—Timmermans and colleagues [28] showed that periconceptional folic acid supplementation (starting prior to conception or up to the eighth week of pregnancy) was associated with decreased risk of preterm birth and LBW, whereas preconception use also reduced the risk for birth of an infant SGA.

On the other hand, a recent meta-analysis has suggested that there are protective effects of maternal overweight or obesity on risk of delivering low birth weight infants, both in developed and developing countries, but especially the latter. However, there was also an increased risk of having an infant of very low birth weight (<1500 g) or extremely low birth weight (<1000 g); the heavier the woman, the higher the risk [29]. This seeming contradiction may be explained by a high risk for malnutrition due to a lack of micronutrients in obese women, as reported by Bodnar and Parrot [30,31].

Finally, it is worth mentioning that a recent study found an association between maternal vitamin D levels at 26 or fewer weeks of gestation and growth measures of newborns. Serum vitamin D levels were positively related to birth weight and head circumference, and negatively associated with the risk of an infant being born SGA [32].

B Other Risks

In addition to the risk of developmental disabilities subsequent to preterm delivery or LBW, intellectual disability (ID) in children has long been linked to known maternal nutrition-related conditions such as those described below.

1 Iron

ID of unknown cause, including autism spectrum disorders (ASD), has been found to be higher in children of mothers with anemia [33]. In 2002 data from 2865 children with ID of unknown cause (grouped into mild to moderate, $n = 2462$; severe to profound, $n = 212$; and ASD with ID, $n = 181$) and 236,964 children without ID were studied. In this study mothers with anemia ($n = 1101$) were five times more likely (odds ratio (OR) = 5.26) to have a child with severe ID (IQ <35 to

40), even after socioeconomic variables were introduced into the stepwise logistic regression model (OR = 4.93). In a large population-based study of children in Hungary with ($n = 781$) and without ($n = 22{,}843$) Down syndrome, the investigators found a lower incidence of Down syndrome among mothers given supplementation of large doses of iron (150–300 mg/day of ferrous sulfate) and folic acid (6 mg/day) during the first month of gestation [34], although only iron was protective when given alone. Note that iron and folate, two nutrients related to LBW, are both depleted in pregnancy and must be replaced [19].

2 Iodine

Iodine deficiency remains the leading cause of preventable ID, including its most severe manifestation, cretinism, and has been identified with other risks during pregnancy such as miscarriages and infant mortality as well as maternal and fetal goiter [35]. The need for iodine increases during pregnancy from an RDA of 150 ug/day to 220 ug/day (RDA for lactation is even higher, 290 ug/day). However, low urinary iodine levels indicate that at least some pregnant women in the US are not currently consuming sufficient iodine. This is likely due to public health efforts to reduce overall salt intakes as well as the increasing preference for sea salt or kosher salt, neither of which contain iodine. Furthermore, according to one study, nearly half of the prenatal supplements marketed in the US contain iodine [36] and that only an estimated 20% of women use supplements that contain iodine [37].

3 Omega-3 Fatty Acids

The toxic effects of mercury on the developing fetus are well known [38], and the US Department of Health and Human Services has recommended limiting seafood consumption by pregnant women because of its high mercury content. This recommendation was revised upward in June, 2014 to at least 8 and up to 12 ounces of fish (2–3 servings) with lower levels of mercury/week in order to support fetal growth and development [39]. This recommendation followed research findings indicating that restricting seafood intake resulted in adverse neurodevelopmental outcomes in children, presumably due to a deficiency in omega-3 fatty acids which have been shown to have positive effects on neurodevelopment [40,41], whereas higher intakes had no ill effects [42]. These findings have been supported more recently by a large study in the Seychelle Islands where fish consumption is high [43], as well as a recent review of the evidence [44]. Fish are also a good source of iodine, choline, zinc, and vitamins A, D, and B_{12}, all important for optimal development. A concern at present is that many women in the US have a low consumption of omega-3s [45], and moreover they are not eating much fish. Using data from 1000 pregnant women, an FDA analysis found that 21% ate no fish in the previous month, and those who did ate less than recommended in the 2010 Dietary Guidelines, with 50% eating fewer than 2 oz a week, and 75% eating fewer than 4 oz [39]. Another concern is the decrease in the availability of fish in the US, from 16.5 pounds/capita in 2006, to 14.4 pounds in 2012 [46].

4 Paternal Nutritional Status

Current research interest is also turning to the role of the father's nutritional status/diet in influencing pregnancy outcomes, reflected in identified paternal epigenetic contributions in animal models [47,48]. Evidence that paternal epigenetic influences are present in humans as well is also beginning to accumulate. Donkin and colleagues [49], e.g., have demonstrated epigenetic changes in human spermatozoa in obese men following bariatric surgery; they postulate that these phenomena might explain earlier observations that the risk for obesity in offspring of obese men is increased independent of the weight of the mother.

C Spina Bifida and Other Birth Defects

The recognition of a relationship between nutrition and the etiology of certain developmental disabilities has been most fully realized in the case of spina bifida and other neural tube defects (NTDs). Although the link between diet and NTDs was suspected as early as the 1960s [50,51] and demonstrated repeatedly in studies that implicated vitamins and minerals in general [51], and gradually focused on folate in particular [52], there was no public health campaign in the US to increase folate intake until the mid-1990s after the publication of the Medical Research Council's definitive study. Then, rather than directing efforts toward increasing women's intake of folate-containing foods, the emphasis was on folate supplementation for pregnant women followed by the passage of the mandatory fortification of cereal grain products in the US that went into effect in January 1998. The latest CDC data show the estimated prevalence has decreased from 10.7 per 10,000 live births in 1995–6 (prefortification) to 6.5 per 10,000 in 2009–2011 (postfortification) and that about 1326 fewer infants have been born over this three-year period with NTDs [53].

The report from the Center on Birth Defects and Developmental Disabilities (CBDDD) noted that the decrease is less than predicted from research trials [54], which led to the Healthy People 2010 goal of a 50% reduction [53]. The Healthy People 2020 goal, MCH 28-1, is a more modest 10% improvement in the incidence of spina bifida using a baseline of 34.2 live births or fetal deaths/100,000 live births [55] and a target of

30.8/100,000 live births [56]. It is worth noting that the incidence of spina bifida is not equally spread among racial groups. Hispanic babies are affected disproportionately (41.7/100,000 live births) compared to either Non-Hispanic Blacks (26.4/100,000) or Whites (32.2/100,000) [55].

The US Public Health Service and CDC recommend a folic acid intake of 0.4 mg/day (supplements or fortified foods) and also a diet that includes folate-rich foods for women of reproductive age [54,57]. There is also evidence that folate, in combination with other vitamins, may play a role in preventing urinary tract defects and congenital hydrocephalus, according to a meta-analysis [58].

Further evidence for the importance of micronutrients other than folate has come from data collected by the Hungarian Periconceptional Service between 1984 and 1994 [59]. In an RCT to test the efficacy of a multivitamin supplement containing folate (0.8 mg) in the prevention of NTDs, researchers were surprised to find that not only were NTDs reduced, but there was a very significant reduction (21 per 1000 population versus 41 per 1000 population) in the incidence of other major anomalies as well, notably cardiovascular anomalies and urinary tract defects [18,60]. This may explain the slow reduction in incidents past the initial period of fortification. A group of investigators in the Netherlands reported significantly lower dietary intakes of iron, magnesium, niacin, fiber, plant protein, and polysaccharides in mothers of infants with spina bifida ($n = 106$) compared to controls ($n = 181$), independent of periconceptional folic acid use [61]. For example, it has been suggested that choline, the dietary requirements for which were set in 1998 [62], is another nutrient that, along with folate and B12, may increase the risk of NTDs when inadequate in the diet. This is because, like folate and vitamin B12, choline has an important role in the methionine cycle, crucial to brain development [63]. Although these and other anomalies are not considered developmental disabilities per se, there also is evidence of an association between the occurrence of orofacial clefts (cleft lip or palate or both) and maternal nutrition as well as a rationale for the potential contribution of several nutrients (folate, niacin, thiamine, B_6, B_{12}, riboflavin, zinc, amino acids, and carbohydrate) to their etiology [64].

Our current understanding of the role of folate in the pathogenesis of NTDs awaited the unraveling of the mystery of the human genome and the development of the field of epigenetics. The research on DNA, RNA, and human chromosomes tells us that the genome, or the inheritable information held in the chromosomes, is not immutable but may be affected by epigenetic markers that interact with other components of the genome. Nutrigenomics, or nutritional genomics, introduces a molecular approach that integrates genomics, nutrition, and health, and was recently reviewed in an Academy Position Paper [65]. The science focuses on the differential influences that nutrients have on the regulation of gene expression by way of nuclear receptors that regulate processes such as embryonic development and cell differentiation and proliferation [66]. It is thought, in the case of folate, because of its role in the methylation of DNA, that a deficiency occurring during the periconceptional period can lead to epigenetic alterations in the DNA of the embryo and to NTDs in the fetus of those women who are genetically susceptible and/or whose need for folate is elevated. Interestingly, the gene for the enzyme methylenetetrahydrofolate reductase (MTHFR), important in the methylation cycle, has a polymorphism that is common in Mexico (32%) but has a very low frequency among African Americans, paralleling the racial demographics of the prevalence of spina bifida alluded to previously [67]. It is certain that nutrigenomics holds future promise in improving maternal and child health and decreasing the incidence of developmental disability (DD).

Another food-related factor that has been implicated in the high incidence of NTDs among Mexican Americans is fumonisins, a class of mycotoxins produced by mold, which interfere with folate metabolism. In response to an "outbreak" of NTDs along the Texas–Mexico border in 1990–1991, an epidemiological study linked it to high levels of fumonisins in the corn used to make tortillas, and other corn products, which form a large part of the diet of this population [68]. Other risk factors, such as folate intake (dietary plus multivitamin), were not confounders. In fact, vitamin B_{12} levels have been shown to be a more important predictor of risk in this population than folate levels [69], and low maternal B_{12} status has been shown to be an independent risk factor in a folic acid-fortified population in Canada [70].

D Fetal Alcohol Syndrome

Fetal alcohol syndrome spectrum disorders (FASD) include fetal alcohol syndrome (FAS), alcohol-related neurodevelopmental disorder (ARNDD), and alcohol-related birth defects (ARBD), depending on the symptoms, and this is an example, according to the life course perspective, of "timing," where the impact of a negative environmental factor is irreversible. The spectrum is defined by pre- and postnatal growth disturbances, dysmorphic facial features, and ID; behavioral abnormalities are common, as are motor problems, facial anomalies, and cardiac defects [71]. The CDC has estimated that the prevalence of US women aged 15–44 years who are exposed to alcohol during pregnancy is 7.3% overall, and highest among married women aged 20–29 [72]. FASD

represents an example of a preventable DD in which prenatal nutrition likely plays a major etiological role, although this is not universally recognized; the recent CDC report does not mention nutrition as a risk factor, for example [72]. However, there are two major reasons for which nutrition should be considered. First, the dietary energy provided by alcohol (7.1 kcal/g or 29.7 kJ), replaces foods containing essential micronutrients, thereby putting the mother and fetus at risk for nutrient deficiencies, and second, alcohol interferes with the metabolism of all fat- and water-soluble vitamins, especially folate, and the mineral zinc [73,74]. These nutrients are key to fetal development because of their involvement in multiple systems: enzyme, gene transcription and regulation, and transport. Folate insufficiency, mentioned previously in the etiology of spina bifida and cleft lip and palate, has also been implicated in the etiology of limb and heart abnormalities and Down syndrome [73]. Severe zinc deficiency has been linked to intrauterine growth retardation and teratogenesis; mild to moderate deficiency has been associated with congenital malformations, LBW, and preterm delivery [73,75]. In recognition of the importance of folate and zinc to reproduction, Young and his colleagues [76] have recently explored the potential nutritional etiology of FAS in a review of current research, using animal models, into the effects of alcohol on folate and zinc metabolism, as well as other nutrients essential to favorable pregnancy outcomes (vitamin A, DHA, choline, vitamin E, and selenium) in relation to FAS. These authors postulate that providing nutrient supplementation to women who drink during their pregnancy might prevent, or at least mitigate, the severity of the disorder [76]. This notion that adequate nutritional status in these women may be protective is supported by observations in a Danish National Birth Cohort study that found effect of mild to moderate maternal drinking (1−8 drinks/week; one drink = 12 g pure alcohol) during early to mid-pregnancy on children's subsequent intelligence, executive function or attention at age 5 [77].

Because of the wide array of nutrients that have been implicated in promoting optimal birth outcomes, it would seem of utmost importance that clinicians and public health professionals urge women of childbearing age to eat a well-balanced diet rather than relying primarily on vitamin and mineral supplements or population-based fortification of foods or food ingredients, as has been suggested by some [78]. In fact, a recent publication by FIGO recommends that practitioners "Think Nutrition First" in order to improve pregnancy outcomes, from preconception to lactation [11]. Furthermore, in recognition of the stresses that a lack of food places on families, as well as the adverse effects on child growth and development, the American Academy of Pediatrics (AAP) has recently recommended that pediatricians routinely screen their clients for food security and access to healthy foods [79].

E Newborn Screening for Metabolic and Other Disorders

Population-based newborn screening (NBS) for metabolic disorders has been established as a preventive public health measure available to all neonates. If infants with specific metabolic, endocrine, and other disorders are identified by NBS in the first few days of life, the diagnostic process can be completed and treatment started before physical and neurological damage occurs. In the case of cystic fibrosis, outcomes (e.g., growth and hospital stays) are improved with the early identification and treatment afforded by NBS. Many of the disorders screened by NBS programs are autosomal recessive. This means that each parent carries the affected gene, and the risk of having an affected infant is one in four for each pregnancy. Thus, affected infants identified by NBS usually do not have a positive family history of the disorder.

Successful NBS of infants depends on reliable, valid, and timely laboratory results. Many states have established a central NBS laboratory, which supports a rigid quality control process. The initial NBS report provides a presumptive positive result that must be confirmed by mandatory laboratory confirmation of the diagnosis.

An expert panel [80] recommends that a NBS blood sample be collected before the infant is discharged from the hospital nursery. If the blood sample is collected before the infant is 24 hours of age, it is essential that a second screening sample be obtained. NBS programs require a small sample of dried blood, which is submitted on filter paper. This type of sample is easily transported to the central laboratory, and many tests can be completed on a single blood sample. Tandem mass spectrometry and other advances in technology have increased the number of disorders that can be effectively screened.

An effective NBS program involves timely community-based collection of initial blood samples, laboratory confirmation, education of primary care providers and families, as well as specific disorder follow-up, management, and evaluation components [80]. Each state has developed individual legislative NBS mandates. However, the Newborn Screening Expert Group, an expert panel, developed recommendations and guidelines for expanded NBS; the report was endorsed by the AAP. The general criteria for inclusion of a disorder in an NBS panel are shown in Table 14.2. Resources for up-to-date information on expanded NBS programs are shown in Table 14.3.

Expanded NBS programs provide the opportunity for earlier presumptive positive identification. Recent laboratory and clinical developments provide an increased specificity in diagnosis and treatment modalities. Thus, the long-term outcome for persons with inborn errors of metabolism and other NBS-identified disorders is brighter than in the past. The contrast between outcomes without early treatment and expected outcomes with early

TABLE 14.2 Criteria for Newborn Screening for a Specific Disorder Used by the Uniform Panel Working Group

Clinical characteristics (e.g., incidence, burden of disease if not treated, phenotype in the newborn)
Analytical characteristics of the screening test (e.g., availability, features of the platform)
Diagnosis, treatment, and management of the condition in both acute and chronic forms (including the availability of health professionals experienced in diagnosis, treatment, and management)

Source: Newborn screening: toward a uniform screening panel and system. Genet Med. 2006;8 Suppl 1:1S–252S.

TABLE 14.3 Newborn Screening Informational Resources

Resource	Description	URL
American College of Medical Genetics and Genomics (ACMG) ACT Sheets and Confirmatory Algorithms	ACT sheets and algorithms developed by experts of the ACMG involved in newborn screening for endocrine, hematological, genetic, and metabolic diseases. The material describes the interrelationships between the conditions screened in newborn screening laboratories and the markers (analytes) used for screening. For each marker, there is (1) an ACT sheet that describes the short-term actions a health professional should follow in communicating with the family and determining the appropriate steps in the follow-up of the infant who has screened positive and (2) an algorithm that presents an overview of the basic steps involved in determining the final diagnosis in the infant.	https://www.acmg.net/ACMG/Publications/ACT_Sheets_and_Confirmatory_Algorithms/NBS_ACT_Sheets_and_Algorithm_Table/ACMG/Publications/ACT_Sheets_and_Confirmatory_Algorithms/NBS_ACT_Sheets_and_Algorithms_Table.aspx?hkey = e2c16055-8cdc-4b22-a53b-b863622007c0 or http://tinyurl.com/pvpa8lh
The National Newborn Screening and Genetics Resource Center (NNSGRC)	Provides information and resources in the area of newborn screening and genetics to benefit health professionals, the public health community, consumers, and government officials; describes the newborn screening program in each state.	http://genes-r-us.uthscsa.edu
American Academy of Pediatrics: Newborn Screening	Compilation of resources related to Newborn Screening	https://www.aap.org/en-us/advocacy-and-policy/aap-health-initiatives/PEHDIC/pages/Newborn-Screening.aspx

identification and treatment are shown in Table 14.4 and discussed further in Section IV.

III THE FUNCTIONAL APPROACH TO NUTRITION ASSESSMENT FOR CHILDREN WITH SPECIAL NEEDS

Special needs vary widely, from conditions that may only mildly affect nutrition status, such as ID, to those with severe and chronic nutritional implications, such as some neuromotor or metabolic disorders. The Nutrition Care Process (NCP) provides a framework for nutrition assessment regardless of the type of special health care need. The NCP is delineated into four steps: nutrition assessment, nutrition diagnosis, nutrition intervention, and nutrition monitoring and evaluation [81]. For this endeavor, the focus will be on the first step.

The assessment portion of the NCP is organized into 5 domains: food/nutrition-related history, anthropometric measurements, biochemical data, nutrition-focused physical findings, and client history. Food/nutrition-related history includes intake reported by the child or caregiver, medication use, and cultural food practices. In pediatrics, anthropometric assessment and nutrition-focused physical findings address growth, body composition, and development, all of which are key in assessing nutrition status. Laboratory findings may identify internal stressors that impact the body's ability to utilize nutrients for growth

TABLE 14.4 Selected Metabolic Disorders that Require Medical Nutrition Therapy (MNT)

Disorder	Pathway Affected	Outcome Without Treatment	Outcome with Early Identification and Treatment	MNT	Supplements/ Medications	Biochemical Parameters Monitored
Disorders of Amino Acid Metabolism						
Phenylketonuria (PKU)	Phenylalanine hydroxylase	Intellectual disability (IQ < 40)	Normal IQ (requires lifelong treatment)	Low phenylalanine, supplemented tyrosine	Potentially, BH4 for some patients	Plasma phenylalanine, tyrosine
Maple syrup urine disease (MSUD)	Branched-chain ketoacid dehydrogenase complex	Encephalopathy → death	Variable; may be cognitively compromised	Low leucine, isoleucine, valine	L-carnitine	Plasma leucine, isoleucine, valine, alloisoleucine
Glutaric acidemia, type 1	Glutaryl-CoA dehydrogenase	Impaired movement, dystonia, vomiting, seizures, coma	No long-term data	Low lysine, tryptophan	L-carnitine; riboflavin	Electrolytes, blood glucose, plasma amino acids
Homocystinuria (HCY)	Cystathionine β-synthase	Cardiac problems, organ damage, psychiatric disturbances, death	Variable; may be physically and cognitively compromised	Low methionine, supplement cysteine	Folate, betaine	Plasma methionine, total and free homocysteine
Isovaleric acidemia	Isovaleryl-CoA dehydrogenase	Metabolic acidosis → coma → death	Variable; may be cognitively compromised	Low leucine	L-carnitine, glycine	Plasma amino acids (isovaleryl carnitine), urine organic acids (isovaleryl glycine)
Tyrosinemia, type 1	Fumarylacetoacetate hydrolase	Liver failure → death	Prevention of neurologic crisis, renal and hepatic failure, rickets	Low tyrosine, phenylalanine	Orfadin (nitisionine)	Plasma tyrosine, phenylalanine, methionine, succinate, alpha-fetoprotein
Disorders of Carbohydrate Metabolism						
Hereditary fructose intolerance (HFI)	Fructose-1-phosphate aldolase B	Hypoglycemia, liver cancer → death	Typical growth, development	Restrict fructose, sucrose		Liver function enzymes
Galactosemia	Galactose-1-phosphate uridyl transferase	Sepsis → severe delays, death	Often learning disabilities	Restrict galactose, use soy formula		Galactose-1-phosphate
Glycogen storage disease, type 1a	Glucose-6-phosphatase	Glycogen stored in liver, severe hypoglycemia, liver cancer → death	Normalization of glucose levels, moderation in liver size, decreased risk of liver cancer	Low lactose, fructose, sucrose; low-fat, high complex carbohydrate	Raw cornstarch, iron and calcium supplements	Cholesterol, triglycerides, uric acid, liver function (AST, ALT, GGT), blood glucose levels

Disorders of Fatty Acid Oxidation						
Long-chain acyl-CoA dehydrogenase deficiency (LCAD)	Long-chain acyl-CoA dehydrogenase	Cardiomyopathy, hepatomegaly, encephalopathy, hypotonia	Variable	Low-fat, low long-chain fatty acids, avoid fasting	L-carnitine, medium-chain triglycerides, thiamine, DHA, glycine	Plasma acylcarnitines, CK, uric acid, liver function studies, CBC, iron status
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	Medium-chain acyl-CoA dehydrogenase	Encephalopathy, hepatomegaly, disability due to severe episodes	No sequelae if no episodes of severe hypoglycemia	Moderate fat, low medium-chain fatty acids, avoid fasting	L-carnitine	If ill, electrolytes, blood glucose
Disorders of Organic Acid Metabolism						
Methylmalonic aciduria (MMA)	Methylmalonyl-CoA mutase or cobalamin cofactor synthesis	Metabolic acidosis coma → death	Variable; may be cognitively compromised	Low protein, isoleucine, methionine, threonine, valine	L-carnitine, sodium benzoate, bicitra	Methylmalonic acid, electrolytes, kidney function (BUN, creatinine), carnitine
Propionic acidemia (PA)	Propionyl-CoA carboxylase	Metabolic acidosis coma → death	Variable; may be cognitively compromised	Low protein, isoleucine, methionine, threonine, valine, long-chain unsaturated fatty acids	L-carnitine, sodium benzoate, biotin, bicitra	Urine organic acids, plasma electrolytes, kidney function
Disorders of Urea Cycle Metabolism						
Ornithine transcarbamylase deficiency (OTC)	Ornithine transcarbamylase	Hyperammonemia → severe delays or death	Variable; may be cognitively compromised	Low protein, supplement essential amino acids, increased energy	L-carnitine, sodium benzoate, sodium phenylbutyrate, L-arginine	Plasma amino acids, ammonia, electrolytes, citrulline, arginine
Citrullinemia (CIT)	Argininosuccinate synthetase		Variable; may be cognitively compromised	Low protein, supplement essential amino acids, increased energy	L-carnitine, sodium benzoate, sodium phenylbutyrate, L-citrulline	Plasma amino acids, ammonia, electrolytes, citrulline, arginine
Carbamyl phosphate synthetase deficiency (CPS)	Carbamyl phosphate synthetase	Hyperammonemia → severe delays or death	Variable; may be cognitively compromised	Low protein, supplement essential amino acids, increased energy	L-carnitine, sodium benzoate, sodium phenylbutyrate, L-arginine	Plasma amino acids, ammonia, electrolytes, citrulline, arginine
Argininosuccinic aciduria (ASA)	Argininosuccinate lyase	Hyperammonemia → severe delays or death	Variable; may be cognitively compromised	Low protein, supplement essential amino acids, increased energy	L-carnitine, sodium benzoate, sodium phenylbutyrate	Plasma amino acids, ammonia, electrolytes, arginine

Sodium benzoate and sodium phenylbutyrate are chemicals administered to enhance waste ammonia excretion; other compounds producing the same effect are also used.

and development, in addition to assessing the functional level of nutrients. Elevated inflammatory markers like C-reactive protein indicate that nutrients might be diverted for healing instead of growth. Children exist within the framework of families and communities. Information about a child's social environment will inform how care is delivered and who needs to be involved.

Levels of nutrition assessment can be categorized from screening (level 1) to more comprehensive but still generalized (level 2) and specialized assessment as part of an interdisciplinary team (level 3) [7]. All three levels of nutrition screening and assessment should utilize the NCP, but the amount and type of information gathered are different depending on the purpose of the interaction and the training of the health care provider. The purpose of screening is to identify children at risk of nutrition problems, to refer children with more severe problems to the next level of care, and to provide anticipatory guidance and educational materials to children and their families regarding the prevention of nutrition problems [7]. Although screening can be completed with information from the caregiver working with health care providers from other disciplines [82], more in-depth nutrition assessment and intervention usually require a registered dietitian nutritionist (RDN), often in conjunction with a specialized interdisciplinary team. For many children with special health care needs, nutrition status will be assessed, monitored, and intervention provided on an ongoing basis by a pediatric RDN (preferably master's prepared) who has been trained in a particular area, such as pulmonary disease, metabolic disorders, or feeding skill development. These dietitians, working with the health care team, utilize their specialized skills to optimize a child's nutritional status in the context of the child's developmental level and medical conditions, as well as the other therapies being provided.

A Nutrition-Focused Physical Assessment

Nutrition-focused physical examination is part of a comprehensive assessment [83]. General inspection of the body for physical findings associated with nutrition status can be found elsewhere [83]. Most physical markers are associated with under nutrition, however there are markers associated with over nutrition like acanthosis nigricans, axillary and abdominal striae associated with metabolic syndrome [84,85]. Growth information is used in nutrition assessment to compare an individual child's growth to that of his or her peers and to evaluate the growth pattern over time. For the information to be valuable, anthropometric measurements must be accurate, the data must be plotted correctly, and the appropriate reference data should be used for comparison [8,86]. At a minimum, weight, height (or length for children who cannot stand independently), and head circumference (especially for children up to 3 years of age) should be measured. Ideally, some form of body composition measurement is also used, with the simplest and most well referenced being skinfold measurements obtained with calipers, which have been shown to be helpful in determining differences in body composition in children with developmental disabilities [87]. Mid upper arm circumference has gained wide acceptance as an indicator of nutritional status for both over and under nutrition and should be included in comprehensive anthropometric assessment [8].

To determine growth rate in children, weight and height for age can be plotted on the WHO growth charts for children 0−24 months and the CDC growth charts for children 2−20 years old [88]. The charts provide a useful reference to monitor weight in relation to stature, including BMI for children older than 2 years. In most cases, BMI-for-age charts are not available for special conditions and have not been validated for use with children with special health care needs whose body composition often differs from that of typically-developing children. For children whose growth parameters fall outside of median values, growth velocity may be a more valuable indicator of nutritional status than percentile of z-score [89]. Tools to calculate z-scores are available online (e.g., www.Pedi-tools.org, www.cdc.gov.growthcharts). Triceps and subscapular skinfolds along with arm circumference measurements can further define the growth and body composition data for the child, although they have also not been validated for children with different body composition. All of these anthropometric data used together can still give a general picture of the nutritional status of the child, whether the child is underweight or overfat, and how linear growth is progressing. In children with neuromotor disorders that make measuring length difficult (e.g., spasticity and contractures), other types of anthropometric measurements have been developed (e.g., knee height or segmental length) with which to estimate stature and linear growth [90], although their clinical usefulness depends on the knowledge and skill of the clinicians obtaining the measurements [91].

Although the WHO and CDC growth charts, which are based on the growth of large numbers of healthy children, can be used to plot growth, some physical conditions affect growth potential. There are specialized charts that may be considered for use with children affected by these conditions. The rationale for using specialized growth charts is clear for genetic disorders where there is evidence that linear growth potential is different. However, the development and use of growth charts for non-genetic conditions, such as cerebral palsy, is complicated by the fact that the underlying cause of the disorder does not change the genetic growth potential. The online training module, "Children with Special Health Care

Needs," is available for a full discussion of these charts (HRSA Growth Charts Training website at depts.washington.edu/growth). Although specialized growth charts may serve as useful references, they have significant limitations. Generally, they are developed from relatively small, homogeneous samples of children with unknown nutritional status, and data used to develop the charts may have been obtained using inconsistent measuring techniques. Also, for children with neuromotor problems, growth can be significantly affected by ambulatory status, as normal growth of the long bones requires weight-bearing; [7] thus using charts specific to cerebral palsy is particularly problematic. One recommendation is to plot the growth patterns of children on both the specialized charts, when appropriate, and the WHO or CDC growth charts [86]. This will allow comparisons of growth to the general population of children and to the references for children identified with a similar condition. Assessing serial growth measurements and using disorder-specific growth charts available for some genetic disorders (e.g., Down syndrome and Prader-Willi syndrome) will help differentiate between normal growth and alterations in growth rate resulting from poor nutrition. To interpret the growth data and understand the causes for unusual growth patterns, further information is needed.

Laboratory assessment of nutrition status is a helpful but less essential part of nutrition assessment for most children. Nutrition information is most commonly derived from plasma, serum, urine, and stool. Despite availability of biochemical assays, cost is a barrier to their consistent utilization. There is no standard battery of laboratory tests recommended for routine screening and assessment in typically-developing children or those with special health care needs. Laboratory tests are performed to confirm or investigate clinical findings. There are some public agencies that obtain laboratory values to screen for nutrition-related concerns. The Special Supplemental Nutrition Program for Women, Infants, and Children routinely collects hemoglobin and hematocrit as a screen for iron deficiency anemia. Some school districts require lead screening prior to entering school. Lead toxicity is associated with anemia and learning differences in young children. The CDC published guidelines that suggest minimal risk with lead levels <5 mg/dL.

B Assessing Dietary Intake

Analysis of a child's dietary pattern can predict and prevent nutrient deficiencies and, as with anthropometric data, requires accurate information to be useful. Data collection techniques may range from screening based on food groups and frequency of consumption, review of a 24-hour recall, or analysis of food records kept for three or more days with the chosen method dependent on the type and accuracy of information needed [92]. New methodologies for dietary assessment include electronic applications that allow participants to input data directly or upload photos that allow for assessment of data. Nutrient adequacy is usually compared to the dietary reference intakes (DRIs) for a child's age and size, unless there are known at risk nutrients [86]. For some conditions, there are known issues of marginal micronutrient status, such as vitamin D status with chronic use of some anticonvulsant medications [93] and for several nutrients when low food intake is a chronic problem (e.g., for some children with cerebral palsy) [94,95].

Dietary analysis will reveal if there are any specific nutrients or groups of nutrients of concern. If anthropometric data provide evidence that the child is overweight or underweight or has altered lean or adipose tissue patterns, this information should be considered along with the dietary assessment in determining the child's energy needs. For children with short stature or who are overweight or underweight, it may be best to calculate energy intake recommendations based on current height (kcal/cm) rather than weight (kcal/kg).

Information relating to other functional areas—elimination patterns, the use of medications, supplements and complementary therapies, the child's ability with varying food textures, feeding skills, and behaviors/interactions of the child and caregiver at meal/snack time should be included as part of the nutrition assessment. The RDN should assess drug-nutrient interactions, fluid intake and output, and the frequency/consistency of bowel movements can indicate if chronic constipation or diarrhea are present, which can compromise feeding behavior and nutrition status.

Often children with special needs have significant feeding skill delays, or feeding patterns that reflect their developmental level rather than their chronological age. Careful evaluation of the type, textures, and quantity of foods consumed is needed to ensure the child is meeting nutrient and energy needs. Food preferences and refusals, which may be related to past or current medical conditions, need to be taken into account when designing dietary interventions. An interdisciplinary team that includes the parent/caregiver, a feeding therapist, the RDN, and a nurse or physician can determine appropriate feeding methods, types/textures of foods, feeding position and equipment, and quantity/type of food to use to facilitate the child's optimal feeding skill development, as well as his or her nutritional intake (see Section IV).

Family-centered care acknowledges the family as the center of the child's existence and has significant relevance in nutrition and feeding. A client history includes information about the family and the household environment, including food security and the family's ability to prepare foods at home. Various caregivers may have

TABLE 14.5 Client History Questions Related to Family and Household Environment

1. Who lives in the home?
2. Who is (are) the primary caregiver(s)?
3. Who prepares the meals in the home?
4. Where does the family eat?
5. Who participates in mealtimes?
6. How would you describe mealtimes? (e.g., stressful, calm, pleasant)

different mealtime expectations and practices that impact a child's mealtime participation. Table 14.5 provides a sample of questions to ask when obtaining a client history.

C Considering Medical Conditions

Medical conditions and/or advancement in medical care can have an impact on nutrition status and growth (shown in Table 14.1). In 2011 it was recognized that children with Down syndrome were living longer and had better quality of life due to medical interventions. For children with Down syndrome, both the UK and US have created updated growth charts that reflect the change in growth status. Key findings of these two recent studies were improved growth in children 0–3 years old compared to the previous growth chart for Down syndrome, which the AAP no longer recommends [96]. Both the UK and the US have created updated growth charts that reflect the change in growth status. The US study also reported increased stature measurements in males 2–20 years old, compared to the previous chart. BMI of those 2–20 years with Down syndrome should be assessed using the CDC growth charts [96–98].

Some conditions (e.g., Prader-Willi syndrome) alter a child's energy and other nutrient needs, whereas other conditions interfere with adequate nutrient intake or utilization (e.g., feeding problems in some types of cerebral palsy). Examples of conditions that increase energy demands are cardiac and pulmonary complications of prematurity and cerebral palsy in which there are significant hypertonia/spasticity or athetoid movements. Other chronic illnesses associated with high energy needs are HIV/AIDS, cystic fibrosis, and mitochondrial disorders. Some genetic disorders are associated with lowered metabolic rates resulting in decreased energy needs (e.g., Down syndrome and Prader-Willi syndrome). Conditions associated with reduced or low muscle mass (as in muscular dystrophies) also result in decreased energy needs. In genetic disorders in which short stature occurs, overall food, nutrient, and energy needs may be lower than those of age peers throughout adolescence and adulthood. If a child has reduced activity levels because of immobility, energy demands will also be lower.

With a few exceptions, such as copper deficiency in Wilson's disease and some metabolic disorders, most medical conditions do not have a primary effect on an individual's micronutrient needs. There is not enough evidence to suggest that nutrients associated with energy metabolism be adjusted even with very high or low energy intakes. Therefore, the DRIs are still the most appropriate guideline for nutrient requirements for children with special needs. In the self-study curriculum "Nutrition for Children with Special Health Care Needs" (available online at http://www.pacificwestmch.org), the following questions are suggested to assess whether and how a medical condition will affect nutritional status:

1. Does the condition (or medication used to treat the condition) have an effect on the child's nutrient needs?
2. Does the condition change the types of foods the child can eat?
3. Does the condition alter the amount of food that the child can reasonably be expected to consume?
4. Does the condition affect the amount of time the child can spend at the table (eating)? Does this make a smaller intake likely?
5. Does the medication or therapy schedule interfere with scheduled meal or snack times?

Consideration of these issues and the impact on overall nutrient intake and nutritional status should be the final component of the nutrition assessment for children with special needs.

IV EVIDENCE-BASED INTERVENTIONS FOR SELECTED CONDITIONS

A Inborn Errors of Metabolism

Inborn errors of metabolism (IEM) are the classic example of the secondary prevention of disability through nutrition therapy. The basic concepts, principles, and strategies of treatment of selected disorders of protein, carbohydrate, and fat metabolism are presented here. A complete discussion of diagnosis and management of the array of IEM can be found elsewhere [80,99]. Table 14.6 outlines the essential components of medical nutrition therapy (MNT) for treatment of IEM. Although the clinical and biochemical presentation of each metabolic disorder is unique and the disorders present a range from mild to life-threatening illness, metabolic disorders can be thought of as a group in which the absence or inactivity of a specific enzyme or cofactor causes the buildup of the substrate and deficiency of the product. The goal of treatment for IEM is to strive for correction of the biochemical

TABLE 14.6 Components of Medical Nutrition Therapy for Metabolic Disorders

Identify precise modifications required for treatment of the disorder
Provide nutritional surveillance to ensure that medical nutrition therapy is adequate
Provide a mechanism for follow-up of child for symptoms of nutritional deficiency or toxicity
Provide emotional and educational support for child and family

abnormality. The outcome of treatment for these disorders is variable and depends on early diagnosis and intensive and continuous intervention. MNT is the primary mode of treating metabolic disorders.

1 Principles of Medical Nutrition Therapy

The two major principles of MNT for IEM are (1) to mitigate the effects of the altered enzyme by modifying components of dietary intake to adjust the environment at the cellular level and (2) to provide protein, energy, and other nutrients to support growth and development.

For many disorders, the treatment is determined by the identification of the missing or inactive enzyme. In an effort to modify the detrimental effect of the decreased or absent enzyme activity, the paradigm of "working around" the enzyme is used. In many cases, decreasing the substrate available for the reaction and supplementing the product to promote "normal" blood levels prevents or decreases the deleterious effects of the disorder. For example, in the treatment of PKU, phenylalanine (substrate) is restricted because of the absence or inactivity of phenylalanine hydroxylase (enzyme), and tyrosine (product) is supplemented. In some disorders, the absent or inactive enzyme is further down the amino acid degradation pathway and may affect the metabolism of two or more amino acids—e.g., leucine, isoleucine, and valine in maple syrup urine disease (MSUD).

The affected amino acids in most disorders of amino acid metabolism are "essential," that is, they cannot be synthesized by the body and therefore must be provided in the diet. These critical essential amino acids (EAAs) must be provided at a level that promotes growth and development but is restricted enough to prevent toxic buildup of amino acid(s) that cannot be metabolized.

In some disorders, the additional step of enhancing enzyme activity by supplying its cofactor can be helpful. An example of this is providing pharmacological doses of biotin in biotinidase deficiency [100] or providing vitamin B_6, on which cystathionine β-synthase is dependent, in some forms of homocystinuria [101]. Use of a synthetic cofactor (tetrahydrobiopterin or sapropterin) for some individuals with phenylketonuria is based on this principle [102].

In metabolic disorders of carbohydrate metabolism, such as galactosemia, the nutrient of concern (galactose) is not essential. Therefore, the goal of effective MNT is to eliminate as much of the exogenous component as possible from the diet [103]. There is no established requirement for galactose because it is produced endogenously. Other sources of nourishment need to be provided to compensate for the omitted foods and their nutrients.

Disorders of fatty acid metabolism require a source of energy other than fat because fat cannot be metabolized to meet energy needs. Fatty acids of specific carbon lengths are often minimized or eliminated, depending on whether or not the fatty acid is essential for growth and development. For example, in long-chain acyl-CoA dehydrogenase deficiency or very long-chain acyl-CoA dehydrogenase deficiency, shorter chain fats can be metabolized and are often supplemented; e.g., medium-chain triglycerides are provided as MCT oil [104]. However, in medium-chain acyl-CoA dehydrogenase deficiency, sources of medium-chain fat should be modestly restricted. Other essential components of treatment are (1) supplementation of L-carnitine, an amino acid that functions in the transport of fatty acid acyl-CoA esters during mitochondrial β-oxidation, and (2) avoidance of fasting because of the accumulation of partially oxidized metabolites associated with impaired energy production [105].

2 Providing Medical Nutrition Therapy

The general principles of protein and energy management that support general nourishment as well as the issues of biochemical control specific to the disorder must be addressed [106]. The infant or child needs to be provided with adequate amino acids, total protein, nitrogen, and energy to support growth. Energy needs may be increased when L-amino acids provide the protein equivalent, and maintaining an adequate energy intake is essential in preventing catabolism. It must also be noted that suppression of destructive metabolites can produce striking biochemical and clinical improvement.

3 Providing Adequate Nourishment

For some disorders, total protein is not restricted, but the composition of the protein may be adjusted. For example, treatment of PKU requires the restriction of phenylalanine intake but not total protein. This is accomplished by using a specially designed semisynthetic medical food (formula). Infants and children with many IEM must obtain most of their protein from specialized metabolic formulas. Natural foods seldom provide more than 25% (often 10%) of the protein requirements of infants and children with amino acid, organic acid, or urea cycle disorders.

Protein in the specialized formulas is provided as individual L-amino acids (excluding those amino acids that are contraindicated for each condition). L-amino acids are more readily oxidized than intact protein; thus, the requirement for protein when L-amino acids are the protein source is greater than usual. Adequate protein and energy are required to maintain an anabolic state. If adequate formula is not prescribed or consumed, a catabolic state will develop, causing both high plasma amino acids levels and clinical problems. Advances in food technology, combined with clinical nutrition research, have produced a naturally occurring protein moiety called glycomacropeptide, refined from whey, which is very low in phenylalanine and has been shown to improve protein retention, phenylalanine utilization, and to reduce hunger when substituted for some of the amino acid formula in the diets of people with PKU [107,108].

The urea cycle disorders require the restriction of overall protein intake because excess nitrogen from any source can be neurotoxic. However, it is also imperative to provide enough protein and energy to support growth. The formulas for the treatment of urea cycle disorders have a high concentration of amino acids to ensure ready incorporation into protein. Most children with these disorders also require medications to enhance the excretion of excess nitrogen through secondary pathways other than the urea cycle. For infants and children with urea cycle disorders, catabolism from excess protein, weight loss, illness, or infection is a danger. Hyperammonemia can occur rapidly (in several hours) and be life-threatening. The total amount of protein tolerated by the individual child depends on residual enzyme activity, age, and growth rate.

4 Supplying Restricted Amino Acids

Small amounts of the restricted EAAs specific to each disorder are required for growth and development. These EAAs are provided to the infant by including small amounts of proprietary infant formula in the metabolic formula mixture. As the child grows and matures, adding small amounts of cow or soy milk or fruits, vegetables, and grains can provide these essential amino acids.

5 Breast-Feeding

Some infants with metabolic disorders are able to maintain low and stable plasma amino acid levels with a combination of breast milk and metabolic formula feedings; e.g., some infants with PKU are able to tolerate partial breast-feeding and maintain low plasma phenylalanine levels. Many infants are too metabolically fragile to maintain appropriate plasma levels with breast-feeding. Breast-feeding is overtly contraindicated for infants with some disorders, such as galactosemia.

6 Feeding

Poor appetite is not uncommon for children with IEM, especially urea cycle disorders, MSUD, and the organic acidemias. Reasons for poor appetite and poor feeding may be organic, behavioral, or both. Many children with IEM who have had acute episodes may also have a history of vomiting, which may lead to feeding avoidance.

7 Growth

Poor growth is often a reflection of several factors: (1) Infants who have endured a severe neonatal illness may require an extended time of appropriate nourishment before they catch up on growth, (2) frequent febrile illness may interfere with achieving expected physical growth, or (3) poor metabolic control or an inadequate protein or energy intake may interfere with growth. Many children with IEM who have little or no enzyme activity are medically fragile, and it is difficult to maintain metabolic balance and support typical growth patterns.

8 Monitoring for Children with Metabolic Disorders

The frequency of monitoring biochemical parameters depends on the age and health status of the child. Maintaining metabolic balance for children with IEM requires frequent and intensive monitoring of biochemical parameters specific to the disorder as well as those that reflect general nutritional status. The goal of treatment for all disorders is to achieve biochemical levels at or near the normal range. Laboratory parameters that are frequently monitored include plasma amino acids, urine organic acids, hematological status, protein status, electrolytes, blood lipids, and ammonia levels. A general plan to guide biochemical assessment is shown in Table 14.4.

9 Specialized Metabolic Team

As a group, children with IEM comprise a small percentage of the pediatric population, and even the most common of these disorders is rare in the general population. However, the health care needs of these children are specific and urgent. Experts groups have recommended that a team experienced in management supervise the therapy of these children [80]. Effective treatment generally requires the expertise of a geneticist, dietitian, nurse, genetic counselor, psychologist, and neurologist. The complex nutritional and medical management of these children cannot occur effectively without the follow-up and support of the community teams. Communication among the team at the tertiary center, the community, and the family is crucial for supporting the best possible medical, nutritional, and intellectual outcome for these children.

B Prader-Willi Syndrome

Prader-Willi syndrome (PWS) is caused by a microdeletion on genes on chromosome 15. Most frequently, this is caused by paternal deletion, some are due to maternal disomy, and about 15% of cases are caused by imprinting defects. PWS is associated with early failure to thrive, hypotonia, abnormal body composition, hypogonadism, short stature, and behavioral and learning issues [109]. Recently seven distinct phases have been identified: [110]

- Phase 0 (in utero): decreased fetal movement, birth weight, and length
- Phase 1: hypotonia without obesity
  - Phase 1a (0−9 months): feeding difficulties with or without failure to thrive
  - Phase 1b (9−25 months): improved growth and feeding
- Phase 2: increased weight
  - Phase 2a (2.1−4.5 years): increased weight without increased appetite or intake
  - Phase 2b (4.5−8 years): continued weight gain, increased interest in food
- Phase 3 (8 years-adult): development of hyperphagia, food-seeking, lack of satiety
- Phase 4: appetite no longer insatiable (has only been observed in some adults)

Delay in identification and treatment of PWS can lead to the onset of gross obesity after infancy.

Abnormal food-seeking behavior is characteristic of PWS and includes hoarding and foraging for food as well as eating items generally considered to be inedible, such as garbage, pet food, or uncooked frozen food. Infants and children with PWS demonstrate a low metabolic rate and thus decreased energy needs. An intake as low as 60−80% of the RDA is often required to maintain a stable weight in older children [109]. Strict and consistent behavioral limits and the establishment of regular routines are helpful behavioral management strategies for food intake and behavioral concerns for children with PWS [111].

Controlled trials have shown that children with PWS benefit from growth hormone therapy from infancy through childhood and into adulthood, with gains in stature and lean body mass. Some studies have also indicated improved lipid profiles. However, growth hormone treatment does not "cure" the underlying causes of short stature in PWS, and it is unclear if the other observed benefits continue after growth hormone therapy is discontinued [109]. New standardized growth curves based on white children with PWS from birth to 36 months of age not treated with growth hormone have been generated [112,113], and charts based on children who did receive growth hormone were recently published [113]. These charts can be used to monitor growth patterns and assess nutritional status for similar infants and children with PWS both treated and not treated with growth hormone.

C Feeding Problems

With increased survival rates of premature and medically fragile infants, there is an increased population of children who are at risk from, or who demonstrate, feeding difficulties with multifactorial origins, depending on the degree of prematurity or LBW and the presence of hypotonia [114]. For example, in premature infants, gastroesophageal reflux, a physiological and developmental consequence of prematurity, may occur three to five times per hour [115]. Poor coordination of the suck−swallow and suck−respiration is often predictive of feeding abnormalities [116]. Feeding problems secondary to oral motor delay occur in 90% of young children with neurological impairment (e.g., cerebral palsy) [117]. Feeding problems may originate from structural anomalies like cleft lip or palate, esophageal strictures, or subglottal stenosis; or neurodevelopmental disabilities such as cerebral palsy, or behavioral challenges due to sensory food aversions [118], or posttraumatic feeding disorder. The range of feeding problems is beyond the scope of this chapter. The multifocal elements inherent in feeding problems requires assessment by an interprofessional team [119] to identify the cause of the feeding problem and prioritize strategies for intervention, which may include medical nutrition therapy.

Nutritional status and feeding development are of primary concern in the neonatal intensive care unit (NICU). Feeding skill development and transitions from parenteral to enteral nutrition in the medically fragile newborn are facilitated by an interdisciplinary team composed of health care providers and the parents. Breast-feeding, or the feeding of breast milk, is encouraged as much as is possible for the newborn to support nourishment, developing attachment, and for feeding skill development. Current recommendations support the use of transitional formula feeds for several feeds in addition to breast milk over fortified human milk, stating that it does not meet the nutritional needs of the VLBW infant [120]. Many infants are discharged from the NICU only after achieving at least a portion of their nutrient intake through oral routes, although the use of nasogastric, orogastric, and gastrostomy tube feedings has increased. Parents of preterm infants reported more problems with early feeding than parents of typical children, and 3-year olds who were born prematurely were more likely to be overweight [121]. If a feeding tube is needed, the plan should encourage developmentally appropriate oral feedings as soon as the child is medically stable and physically able. Early introduction of low volumes (trophic feedings), as early

as 33 weeks' gestational age, has been shown to accelerate the rate at which preterm infants are able to attain full oral feedings [122]. More recently the literature supports the use of standardized feeding protocols to achieve full feeds in VLBW infants [123].

Among children with developmental delays and special health care needs, especially those born prematurely, the estimated prevalence of feeding disorders ranges from 33 to 80%, depending on the definitions of feeding disorders [124–126]. Problems with feeding occur when the components for the development of normal feeding are missing or delayed. Significant behavioral refusals due to multiple etiologies may include discord between the child and caregiver, medical interventions and medications that interfere with appetite and regulation, or physiological problems related to the medical condition or disability (Table 14.7). A recent review classified children with feeding disorders as: (1) children with fear of feeding as a result of adverse events, (2) children with limited appetite due to medications, and (3) children with food selectivity. An extensive discussion of the physiological and behavioral contributors to feeding problems is included in a special issue of *Developmental Disabilities Research Reviews* devoted to this topic [127]. Whereas inpatient behavioral therapy has been shown to be effective for children with feeding interaction problems [126], children with persistent physical challenges resulting in swallowing and other difficulties often require supplemental or total tube feedings for long periods of time and may never become independent oral feeders. Utilizing a sophisticated statistical method (latent class analysis) of chart review data from 286 children referred to a feeding team, Berlin and colleagues [128] demonstrated that children fed by gastrostomy tube had both more behavioral feeding problems and higher weights across all categories sharing common medical, developmental, and behavioral conditions. This group concluded that the presence of multiple comorbid conditions confers general, rather than specific, risk from feeding problems.

An interdisciplinary team that can determine factors leading to feeding difficulties and develop and coordinate appropriate interventions is recommended [125,129]. The role of the RDN on the interdisciplinary feeding team is to assess nutrition status and to determine how best the child can meet his or her nutritional needs using developmentally appropriate feeding methods and forms of foods whenever possible [86,130]. Use of dietary supplements, including nutritionally complete formulas, either orally or through a tube, may be needed to supply adequate energy, protein, and other nutrients. It is often best to use the child's size and developmental stage (e.g., pubertal and prepubertal) to estimate nutrient needs, rather than the child's chronological age.

Nutrition interventions for children with ASD are discussed in the next section, but it should be mentioned here that although children with ASD often have feeding challenges, the diet often meets or exceeds the RDA for protein, carbohydrates, and fat [131–133]. However, rigid mealtime patterns or limited food repertoires are common feeding issues for children with ASD [131,133,134]. For example, the child with ASD may insist on only white foods or demand a particular plate. The child may have a preference for only salty or only sweet foods or only foods of a certain texture. These behaviors may make family meals difficult, and severe food jags can lead to nutrient deficiencies. If entire food groups are missing, or if there is excessive intake of a particular nutrient, a vitamin/mineral supplement may be appropriate. Pica (the eating of "nonfood" items) may also be more common than in the general population [133], and nutrition

TABLE 14.7 Feeding Problems in Children with Special Health Care Needs

Feeding Difficulty	Frequent Causes	Associated Conditions
Delayed or slow development Posturing or seating difficulties Persistence of primitive reflexes Craniofacial anatomic problems Uncoordinated sucking, chewing, swallowing Behavioral refusals Decreased appetite	Hypertonia Hypotonia Hypersensitivity Developmental immaturity (prematurity) Gastroesophageal reflux Unpleasant intrusions into the oral cavity Unpleasant feeding experiences (past or present) Constipation Increased secretions Decreased gastric motility Medications	Developmental delay Cerebral palsy Cleft lip and cleft palate Down syndrome (trisomy 21) Prader-Willi syndrome Rett syndrome Muscular dystrophy Williams syndrome Myelomeningocele with accompanying Arnold-Chiari malformation Autism Chronic respiratory diseases (cystic fibrosis, bronchopulmonary dysplasia, respiratory distress syndrome)

education and intervention may need to address this behavior. Therapies to expand food choices vary and can be difficult to implement. Families can be encouraged to offer new items with preferred foods [135] and to offer them repeatedly, possibly over a period of months. Interdisciplinary intervention is usually necessary, including a behavior specialist, or an occupational or speech therapist where texture or oral issues are also present.

Identifying the need for nutrition intervention early in a child's life—and providing ongoing and coordinated services between inpatient, early intervention, and school- and community-based services—is ideal but often challenging based on the complexity of the health care system and lack of nutrition services available in many settings [5,136]. A practical and detailed description of feeding problems, developmental conditions leading to feeding problems, and suggested feeding and nutrition assessment strategies is included in the self-study curriculum referred to earlier, "Nutrition for Children with Special Health Care Needs," Module 3: Feeding Skills (available at http://www.pacificwestmch.org).

D Autism Spectrum Disorders

1 Definition and Etiology

Autism spectrum disorder (ASD) is a group of disorders characterized by deficits in social communication/interaction and symptoms related to restricted and repetitive behaviors. Previously, the diagnosis included subdiagnoses (Asperger syndrome, autistic disorder, pervasive developmental disorder not otherwise specified, and disintegrative disorder). The fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) established modified diagnostic criteria [137]. ASD is characterized by two key manifestations that are functionally impairing:

1. Persistent deficits in social communication and social interaction across multiple contexts (e.g., problems with the following: establishing or maintaining conversations and interactions, shared attention, adjusting to different social situations, nonverbal communication such as eye contact, expressions, and gestures)
2. Restricted, repetitive patterns of behavior, interests, or activities (e.g., repetitive speech or movement, rigidity and need for routine, restricted interests, hyper- or hypo-reactivity to sensory input)

Intellectual abilities can range from typical to profound ID. Although the reasons are not clear, the prevalence of ASD appears to be increasing in the US; surveillance data from the CDC indicate that 1 out of 68 US children has ASD, and for boys, the estimated prevalence is even higher at 1 in 23.6 [138]. This survey showed that non-Hispanic black and multiracial children were at lower risk of having an ASD than were white children.

The etiology of ASD is unknown, and the diagnostic criteria are currently based solely on behaviors. However it is becoming clear that the etiology is likely to be multifactorial, with involvement of genetic, epigenetic, and environmental factors, which possibly include nutrition. Comorbidities often seen in the ASD population, which may affect feeding or nutritional status, include fragile X syndrome, seizure disorder, anxiety disorders, and gastrointestinal disorders [139]. Currently, there is active research focused on gastrointestinal, nutrition, and feeding issues in children with ASD. Each is briefly discussed next.

2 Gastrointestinal Issues

Although the current diagnostic criteria for ASD do not include gastrointestinal (GI) symptoms, reports since the 1970s describe chronic GI dysfunctions/symptoms, including diarrhea, constipation, abdominal bloating, discomfort, irritability, GI reflux, and vomiting among children with ASD [140], and a meta-analysis found a higher prevalence of GI symptoms among children with ASD [141]. Children with autism were four times more likely to have general GI symptoms, three times as likely to have diarrhea or constipation, and twice as likely to have abdominal pain.

Other studies have reported pathological findings in the GI tract of children with ASD, including increased intestinal permeability and compromised gut microflora [133,142]. Children with ASD who have a limited ability to communicate may be unable to express GI discomfort. GI problems should be considered along with other causes if negative behaviors are present and/or escalate in children with ASD.

A consensus report sponsored by the Autism Forum—a group of 28 participants in seven working groups (including nutrition) and representing 10 disciplines and multiple organizations—identified, graded, and evaluated publications related to GI issues and autism and found a general absence of high-quality research data, which precludes evidence-based recommendations at this time [143]. They developed and published 23 consensus statements, including the following:

- GI disturbances in ASD, likely highly prevalent, are incompletely understood and may be difficult to evaluate because of problems communicating discomfort/pain
- Children with ASD and GI symptoms may have more problem behaviors than others with ASD; these may be a sign that a GI evaluation is warranted

- GI disturbance specific to ASD has not been established; evidence for abnormal GI permeability in ASD is limited, and the studies are methodologically challenged
- ASD caregivers and health care providers need to know how to recognize signs and symptoms of GI disorders and other nutritional problems
- Evaluation by a nutritionist is recommended; anthropometry should be monitored; those with limited diets or taking supplements should be evaluated
- Additional studies are needed before recommending specific diets for ASD; data do not support the use of a casein-free or gluten-free diet (or both) as primary treatment
- Detailed history to identify potential associations between GI and/or behavioral symptoms and allergies; involvement of specialists (GI, nutrition, and feeding therapists) can be beneficial
- Direct relationship between immune dysfunction and ASD is not yet proven, but it warrants further investigation
- Well-defined phenotypes and genotypes will enhance further clinical investigations

Recently, an expert panel developed an algorithm for nutrition management of GI concerns in children with ASD [144]. Their report addressed barriers specific to ASD, including the following:

- Food selectivity
- Complementary-alternative diet therapies
- Nutritional deficits/excesses
- Importance of interdisciplinary approach
- Behavioral response to changes in mealtime routine

Special diets are popular complementary therapies for children with ASD. The theories behind their use are numerous and include GI-related implications, possible food sensitivities and/or allergies, altered immune responses, and altered intestinal microflora. The "opioid excess" theory holds that a "leaky gut" allows undigested opioid-like peptides from the proteins gluten and casein to cross the intestinal wall and then the blood–brain barrier, where they stimulate opioid receptors and disturb brain neurotransmission, causing or increasing behavioral symptoms [132,145]. Although some earlier studies reported increased urinary peptides in some children with ASD, a case-control study found no differences between those with ASD and typically-developing controls [146].

Case studies and anecdotal reports often lead families to choose an alternative diet for their child. Inflammation of the GI tract resulting from exposure to irritants is uncomfortable or even painful. Because most studies use parent or caregiver reports of behavior changes in children after implementing the diet, a significant potential confounder is the placebo effect because parents seeking to find any treatment for their child may be susceptible to suggestion. Also, most studies have not been of a prospective, randomized, blinded design; e.g., they begin with a population of children with ASD who already have GI issues. This makes it difficult to generalize findings to the entire ASD population. Thus, there is controversy around whether the gluten/casein-free diet is a valid treatment for ASD or simply a treatment for comorbid gluten/casein sensitivity in a subgroup of these children. A meta-analysis of 14 studies concluded that published data do not support the use of gluten- and/or casein-free diets for children with autism. Conclusions of randomized controlled trials are mixed. The ScanBrit study (single-blind) suggested that the diet "may positively affect developmental outcome" [147]. However, a more recent double-blind, placebo-controlled challenge trial found no differences in physiologic and behavioral outcomes [148]. Worse, the recommendations for alternative diets may be influenced by profit or ideology (some physicians sell the products they recommend). At this time, therefore, there are no recommended diet therapies for ASD.

The role of the RDN who works with families of children with ASD is to ensure adequate growth and development and to support the family who wants to try an alternative diet. Parents need to understand what is currently known about the efficacy of the diet (based on current evidence) and if there are any potential deficiencies; the more restrictive the diet, the greater the risk. It is important to help families clearly understand the difficulties that may arise in attempting to follow a special diet, especially when a child eats outside of the home on a daily basis (e.g., at school or with other caregivers). At school, eating separately or different foods may further isolate the child from his or her peers. The diet can be more costly than a traditional diet. If the child is a "picky eater" or has feeding problems, these may be complicating factors when introducing a restrictive diet.

3 Nutrition Issues

The growth patterns of children with ASD are thought to be normal. However, studies have shown an increased rate of head growth in children with ASD [149]. Others have shown an increased rate of linear growth, weight gain, and body mass index [150]. Anthropometric data for this population should still consist of length, weight, weight/length, and head circumference. When the child is found to have a large head size, this should be considered when making weight/height comparisons because they could be deceptively increased. In children with ASD, it is not unusual to see a very low or very high BMI as a result of feeding issues.

Complementary therapies are very popular; dramatic testimonials abound, and there is an active promotion of alternative and diet therapies in books, on websites, and at autism conferences attended by parents. Of more than 3000 participants in the Autism Speaks Autism Treatment Network (ATN) registry, 28% reported use of a complementary or alternative therapy (CAM), with 17% using special diets and 20% using another form of CAM. CAM use was higher among children with GI symptoms, seizures, and higher scores on a behavior problem rating scale [151]. Given the lack of scientific evidence, the question is why do parents choose dietary therapies? Answers include: (1) frustration with the limitations of current therapies coupled with hope for a cure or at least an improvement in behavior, (2) comfort in knowing parents have done everything possible for their child, and (3) wanting to have some control over the treatment. Some parents prefer "harmless" treatments over drugs. Finally, parents may give the therapy credit for changes that would have occurred anyway. The greater the efforts, the more biased they may be toward seeing them "pay off."

Potential nutrition problems with the casein-free, gluten-free diet—an example of a popular diet—include risks associated with the avoidance of casein such as insufficiency of vitamin D and calcium [152]. Hediger and colleagues found decreased cortical bone in males with ASD [153]. Milk is often a major source of protein for children as well. The elimination of gluten leads to a risk of inadequate sources for B vitamins in the diet. Vitamin/mineral supplementation for these children may not be appropriate, sufficient, or safe (not regulated). For example, children's multivitamins typically lack enough calcium and vitamin D to make up for the lack of milk in the diet; the popular chewable children's "gummy" brand formulations lack iron. And, even if an appropriate supplement is identified, a child with an extremely limited food repertoire might not accept it.

The ATN is a national research project (17 sites throughout the country) that is studying and establishing the medical standard of care for ASD. Included in this effort are several nutrition-related studies to describe the nutritional status of children with ASD, their associated medical and behavioral symptoms, and investigate any relationships among dietary intake, feeding behaviors, and food preferences of the children and their families [154]. One report of the Diet and Nutrition Study of the ATN found that children with ASD had similar intakes to controls—neither group met recommended intake levels for fiber, choline, calcium, potassium, and vitamins K and D. Supplements were used by 66% of children with ASD [131]. This study underscored the need for nutritional surveillance for children with and without ASD, and the authors recommended dietary assessment corroborated with anthropometric and laboratory data.

Currently, risperidone is the only FDA-approved medication for treating symptoms associated with ASD. Other medications are also sometimes used to treat specific ASD symptoms. Drug-nutrient interactions should be assessed if children are prescribed drugs to treat comorbidities such as seizures or psychiatric symptoms. The most common drugs used in this population that have significant drug-nutrient interactions have been reviewed by Geraghty and colleagues [140]. They include stimulants used for ADHD (Concerta and Strattera), which may cause a decrease in appetite and also nausea, vomiting, constipation, and diarrhea in the case of Strattera. Another category of commonly used medications are anticonvulsants (Depakote and Keppra). Depakote may also decrease appetite with associated nausea, vomiting, and diarrhea and also decrease serum vitamin D and calcium. The antipsychotic drugs (Zyprexa and Risperdal), on the other hand, may increase appetite and cause weight gain, at least in the case of Zyprexa.

Finally, it has been suggested that some nutrient deficiencies, or relative deficiencies, have been implicated in the etiology of some of the behavioral symptoms of autism. Vitamin B_6 is an example because of the role it plays in neurotransmission and based on evidence that there is a lack of an enzyme needed to convert it to its active form. To date there is not enough evidence to support the recommendation for vitamin B_6 supplementation as a treatment for ASD [155].

4 Feeding Issues

Many children with ASD have hyper- or hypo-reactivity to sensory input, which may include taste, smell, and oral sensitivities. These sensory issues may be responsible for the high prevalence of problem eating behaviors seen in children with autism [156,157]. These behaviors may include selective food refusals such as textures, colors, and food groups [134,157]. Other selective behaviors affecting feeding may relate to the GI issues referred to previously, fear of new foods, or obsessive mealtime rituals such as the need for sameness. The child with ASD may also have difficulties with changes in the environment [158], which may lead to problems with school mealtimes where a loud and boisterous cafeteria may be overwhelming such that a child with ASD may have difficulty eating lunch with a group of peers.

V CONCLUSION

In this review, we have provided examples of nutrition issues related to the role of nutrition in the primary (maternal nutrition) and secondary (MNT for IEM) prevention of developmental disabilities, and in the secondary prevention of nutrition-related disorders that are

commonly seen in children with special health care needs. Emphasis was given to several important concepts. First is the growing evidence regarding the contributory role of nutrition to the life course trajectory, through epigenetic and nutrigenomic relationships as yet not fully understood, in the etiology of preterm birth, low birth weight, and some birth defects, as well as the essential role of nutrition in early postnatal development. Because of the growing numbers of nutrients shown to be involved in normal development, the importance of whole foods versus supplements is also becoming apparent. The second major point, which follows from the first, is that early nutrition intervention is crucial to the individual child's attaining his or her highest potential and optimizing human development throughout the life course. Third, we presented a functional approach to assessment, early detection, and treatment of nutrition problems in children with special health care needs, stressing the point that although diagnoses may differ, the nutrition issues are often similar. Finally, although we did not cover the importance of training dietetics professionals in this area or the need to improve families' access to nutrition services for children with special needs, we refer the reader to the latest position paper of the Academy of Nutrition and Dietetics for an excellent review of the legislative history and a presentation of the challenges that remain for the profession [5] as well as another recent paper [159].

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Chapter 15

Bioavailability and Metabolism of Bioactive Compounds From Foods

Andrew P. Neilson[1], Katheryn M. Goodrich[1] and Mario G. Ferruzzi[2]
[1]Virginia Polytechnic Institute and State University, Blacksburg, VA, United States, [2]North Carolina State University, Kannapolis, NC, United States

I INTRODUCTION

A Dietary Phytochemicals

Plants produce a variety of compounds that exert biological activities in humans and animals. Bioactive components in edible plants are of interest for disease prevention, as their widespread use with minimal toxicity has the potential to impact human health on the individual and population level. The majority of plant bioactives are secondary (2°) metabolites, as opposed to primary (1°) metabolites. The 1° metabolites are required for the biochemical processes of cells (such as amino acid, energy, and nucleic acid metabolism), while 2° metabolites are not required for survival of the organism that produces them. Rather, 2° metabolites are specialized compounds which confer added survival and competitive advantages to the plant. Plant 2° metabolites are referred to as natural products as well as phytochemicals (although "phytochemical" technically refers to any plant metabolite).

Major classes of plant phytochemicals are the phenolics/polyphenols, alkaloids, and terpenoids. Minor classes include polyacetylenes, polyenes, miscellaneous pigments, cyanogenic glucosides, glucosinolates, and nonprotein amino acids. Refer to Table 15.1 for chemical properties of major classes. The majority of the dietary bioactives associated with disease prevention are phenolics or terpenoids.

Dietary phytochemicals are often divided into two distinct classes based on their structure, solubility, and physiological absorption properties: water-soluble and lipid-soluble. In terms of relevance to diet and disease, the principal water-soluble phytochemicals in the diet are the phenolics, and more specifically, polyphenols. The principal dietary lipid-soluble compounds with potential health benefits include the carotenoids, tocochromanols (vitamin E derivatives) and curcuminoids. Although curcuminoids are technically phenolics, their hydrophobicity and digestive behavior align them more with lipid-soluble compounds for the purpose of this chapter.

Dietary phytochemicals have a variety of roles in nutrition and health. Some (such as certain carotenoids, through provitamin-A activity, and tocochromanols, with vitamin E activity) are essential human nutrients required for development, maintenance, and health. They may also exert benefits beyond basic nutritional value, including reduced risk of chronic diseases. Conversely, nonnutrient (i.e., nonessential) phytochemicals are considered xenobiotics: chemicals consumed by an organism, but which are not normally produced by the organism (metabolites from the organism's own biochemical pathways) and are not expected to be present in the organism or used for normal metabolic function (nutrients). The term xenobiotic does not imply either harmful or beneficial biological activity, but rather denotes that the compound is regarded as foreign to the body and is metabolized and excreted as such. This commonly includes pharmaceuticals, pollutants, and natural products such as phytochemicals all of which may be toxic, beneficial, or neutral. A growing body of evidence in medicine, biochemistry, nutrition, and epidemiology supports the role of both dietary nutrients and xenobiotic phytochemicals in the prevention of disease (Table 15.2) [1–17].

B Definition of Bioavailability

The bioavailability of dietary phytochemicals is a measurement of their distribution in body fluids and exposure to tissues. Ultimately, bioavailability is critical for

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TABLE 15.1 Properties of Plant Natural Products

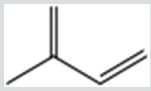
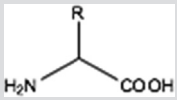
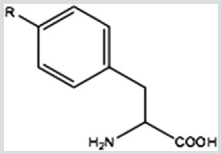
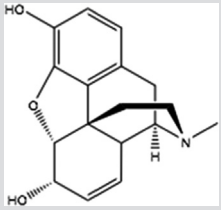
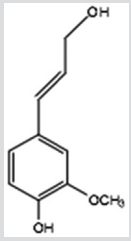
	Natural Product Class		
	Terpenoids	**Alkaloids**	**Phenolics**
Biosynthetic precursor	 Isoprene	 Amino acids	 Phenylalanine, tyrosine
Chemical nature	Hydrocarbon	Nitrogen-containing	Phenol ring(s)
Characterized compounds	>25,000	>12,000	>8000
Example	 (–)-Limonene	 Morphine	 Coniferyl alcohol

TABLE 15.2 Reported Roles of Dietary Phytochemicals in Basic Health and Disease Prevention

Solubility	Class	Functions as Essential Nutrients	Putative Functions in Prevention of Disease
Water-soluble	Phenolic acids	None	Inflammation, cancer, vascular function and cardiovascular disease, general antioxidant activity
	Flavonoids	None	Inflammation, cancer, vascular function and cardiovascular disease, metabolic syndrome (obesity, diabetes, low-grade inflammation), neuroprotection, general antioxidant activity, modulation of colon microflora, and immune stimulation
Lipid-soluble	Carotenoids	Eye health and vision, cell growth and differentiation, cell signaling, reproduction, immune function	Cancer, cardiovascular disease, osteoporosis, skin health, general antioxidant activity
	Tocochromanols	Antioxidant activity, membrane function, immune function	Cardiovascular disease, neuroprotection, general antioxidant activity
	Curcuminoids	None	Inflammation, cancer, neuroprotection, skin health, general antioxidant activity

bioactivity, because exposure to target tissues determines the potential of these compounds to deliver beneficial activity. A standardized definition of bioavailability for pharmaceutical agents is defined in the United States as follows: "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action" (21 Code of Federal Regulations 320.1). This definition can be modified to apply to bioactive phytochemicals from the diet (foods, supplements, etc.): "bioavailability is defined as the rate and extent to which the phytochemical or active moiety is absorbed from the

ingested matrix and becomes available at the site of action." Essentially, this definition results from the two factors governing the activity of any compound in vivo: (1) the active compound must be present at the site of action to impart its biological activity, and (2) the concentration and the length of exposure of the active compound at the site of action determine the magnitude of activity.

Interest in the biological activities and resultant health-promoting and/or health-protecting functions of plant foods and their constituents has resulted in research endeavors to elucidate the factors that impact the bioavailability of dietary phytochemicals. The aim of this research is primarily to identify food and dietary factors (macro- and microcomposition, physical form, phytochemical concentration, dose, and exposure rate) and physiological factors (digestion, absorption, metabolism, distribution, and excretion processes) that alter bioavailability. Recently, disease conditions including obesity and diabetes have been identified as factors that may impact absorption and metabolism of dietary phytochemicals. Such knowledge is then used to optimize the bioavailability of dietary phytochemicals, and by extension, their activities at the tissue of interest.

It should be noted that the definition of bioavailability refers to the availability of one or a few specific compounds at their known or desired site of action. This definition is more easily applicable in its purest sense to pharmaceuticals than to phytochemicals originating from complex food matrices. Pharmaceuticals are comprised of one or a few active ingredients with rigorously established activity, and are designed to exert a specific function in a specific tissue in a specific "intent to treat" population (age, gender, disease state, etc.), with a specific indication of an existing or likely disease (an indication is a symptom or cause which suggests the proper and efficacious treatment). Foods and supplements generally do not meet these criteria, complicating the utility of bioavailability as a means to optimize the efficacy of dietary compounds in several ways.

First, with the exception of deficiencies in essential nutrients, definitive science on the relationships and exact mechanisms linking specific diets and/or phytochemicals to the prevention or amelioration of diseases continues to evolve. Dietary phytochemicals, other dietary components (fiber, toxins, pesticides, etc.), genetic factors, environmental factors, age, gender, and lifestyle all contribute to the interaction between diet and disease risk.

Secondly, foods and dietary supplements typically contain hundreds, if not thousands, of phytochemicals with diverse and largely unknown biological activities. The biological activities of foods and supplements are likely due to the combined effects of numerous phytochemicals including additive, synergistic, and/or antagonistic activities, rather than a single compound or small group of compounds. This is a potential obstacle for bioavailability research, as it is both desirable and practical to measure only a small percentage of the compounds ingested. Typically, bioavailability studies select the phytochemicals to be measured based on three main criteria: (1) predominance of the compound(s) in the dietary source to be studied (which is problematic, as abundance does not necessarily indicate activity), (2) epidemiological or other evidence suggesting a link between consumption of the phytochemical(s) or foods rich in the phytochemical(s) with the biological outcome of interest, or (3) in vitro or in vivo studies suggesting that the phytochemical(s) provide the desired biological outcome when administered in purified or semipurified form.

Thirdly, the observed biological effect of a phytochemical is not necessarily due to the native form found in foods. A variety of metabolic processes that occur during and postabsorption can chemically transform the phytochemical into metabolites. These systems break down native dietary phytochemicals into smaller compounds (via gut microbiota preabsorption) and alter their functional groups (phase-I and -II detoxification systems postabsorption). Therefore, these metabolites may, in fact, be the putative compounds responsible for some or all of the observed biological activities in vivo. However, many studies measure the bioavailability of the native phytochemicals, while ignoring the metabolites, for several reasons: (1) most phytochemicals can be converted into many metabolites, which exponentially increases the number of compounds to measure, (2) the profile of metabolites arising from a single phytochemical is often unknown or incomplete, (3) the activities of the native compound are better characterized both in vivo and in vitro, and (4) the native compound serves as a marker for all of its metabolites (albeit an imperfect one). Furthermore, many phytochemicals, particularly phenolics and polyphenols, can be broken down into a similar or even identical suite of metabolites, meaning that distinct native compounds may produce similar bioactive compounds.

Finally, the actual disease-modulating activity of a particular dietary compound may occur in different known and unknown sites in the body. Furthermore, the site where the compound exerts its activity need not be the site of the desired effect. Stimulation of sensory, neurological, endocrine, and immune function by the active compound at one site may result in a significant biological response at another distant site where the active compound either is not present, or is present but does not produce the observed effect locally. Therefore, selection of the site at which bioavailability is to be determined is critical for accurately assessing phytochemical delivery with relevance to specific biological outcomes. Similarly, benefits of phytochemicals are often associated with

population studies that link chronic (multiple doses over extended time periods) dietary patterns of food rich in phytochemicals. However, bioavailability of phytochemicals from foods is typically assessed using only acute (single dose) experimental paradigms that are not reflective of common dietary practices or exposure. Also, bioavailability studies measure the concentration of the phytochemicals(s) of interest in one of several blood fractions (plasma, serum, lipoprotein fraction or whole blood). This has several advantages: (1) it provides a measure of general systemic availability, (2) blood is readily accessible in a healthy clinical study population and does not require highly invasive collection procedures, (3) data from blood bioavailability are in some cases representative of a wide variety of tissues. Blood bioavailability, however, has the disadvantage of not being specific to the desired organ or tissue of interest (unless blood or the circulatory system, i.e., the vasculature or heart, is in fact the site of interest) and may not be representative of chronic dietary exposure for rapidly cleared compounds. Several tissues where venous blood from an extremity is less than ideal for assessing bioavailability include the brain (due to the high selectivity of the blood−brain barrier), kidneys (urine may be a better marker), adipose tissues (where lipid-soluble compounds may accumulate over chronic exposure), and the intestines (due to flow of absorbed dietary components from the intestines to the liver prior to entering systemic circulation). These limitations should be considered when planning and interpreting bioavailability studies.

C Measurement of Bioavailability

The bioavailability of dietary phytochemicals is commonly assessed through characterization of their acute absorption and distribution, otherwise termed "pharmacokinetic (PK) behavior," following ingestion. PK refers to the kinetics (concentration with respect to time) of the phytochemical's appearance in and subsequent clearance from circulation, typically measured in a desired blood fraction. Typically, PK data are obtained by measuring the concentration in a blood fraction following an acute dose at several time points, starting with baseline (time of ingestion, where concentration is essentially zero), and continuing until the compound has been cleared from circulation (concentration returns to zero). Studying PK from baseline to clearance is more accurate and less biased than collecting data at a single time point following acute or chronic dosing. PK parameters may then be calculated from the concentration-time data based on several distinct mathematical models. Bioavailability can also be assessed by quantifying delivery to a specific target tissue, as opposed to blood, as a function of time. However, most tissues are difficult to collect for these purposes, due to the destructive and invasive nature of tissue sampling.

PK calculations and comparisons are of interest as a means to quantify phytochemical availability for delivery to target tissues and subsequent bioactivity and the adaptability of these parameters to chronic exposure or under specific disease conditions. While PK calculations can be complex, typically four main parameters are observed in the literature: C_{MAX}, T_{MAX}, AUC, and $t_{1/2}$ (Fig. 15.1). C_{MAX} refers to the maximal concentration obtained in the blood fraction during the specified experimental period. The magnitude of C_{MAX} indicates the maximal concentration to which tissues may be exposed; higher C_{MAX} values are commonly desirable for improved delivery to tissues with additional endothelial barriers such as brain. T_{MAX} refers to the time at which C_{MAX} was observed. Smaller T_{MAX} values indicate more rapid absorption and appearance in the blood. AUC (area under the curve) represents the calculated area (in units of concentration × time) under the concentration (y-axis) versus time (x-axis) curve. AUC is typically the best comprehensive measure of bioavailability (total systemic exposure), as it reflects both concentration and time and accounts for the entire shape of the PK curve. The elimination half-life, $t_{1/2}$, reflects the time required for 50% clearance (excretion and/or degradation) from the blood fraction (calculated as the time between C_{MAX} and ½ C_{MAX}). Smaller $t_{1/2}$ values indicate rapid clearance from the blood, which may limit exposure and efficacy. These parameters are used to quantify the bioavailability and clearance of selected compounds of interest from circulation. The parameters above reflect absolute bioavailability (where bioavailability is assessed by a single delivery method). For dietary phytochemicals, absolute bioavailability is assessed by oral administrations. Another

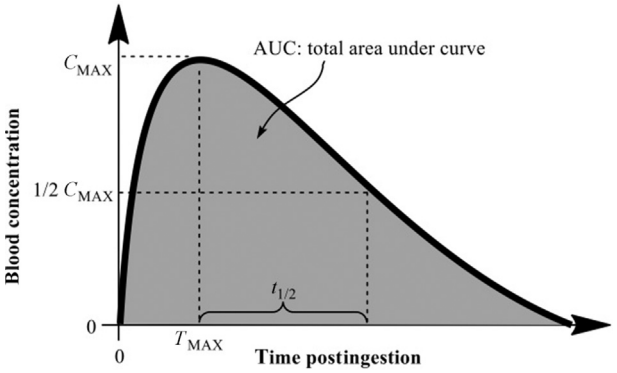


FIGURE 15.1 Illustration of PK parameters used to assess systemic bioavailability of dietary bioactive compounds: C_{MAX} (the maximal concentration obtained in the blood), T_{MAX} (the time at which the maximal blood concentration occurs), AUC (area under the concentration × time curve), and $t_{1/2}$ (the elimination half-life, which is the time required for 50% clearance from the blood).

parameter of interest is relative bioavailability, determined as a ratio by comparing the AUC or C_{MAX} values obtained by two different administration routes. Typically, bioavailability from oral administration is expressed relative to that obtained from intravenous (i.v.) injection. Oral bioavailability is typically lower than i.v. bioavailability, as i.v. injection bypasses several limiting processes (digestive release and solubility, etc.), absorptive barriers (intestinal epithelium), and metabolizing tissues (first-pass metabolism by intestinal epithelium and liver) encountered by orally delivered phytochemicals.

II BIOAVAILABILITY OF WATER-SOLUBLE COMPOUNDS

A Polyphenols

Polyphenols are a structurally diverse group of compounds that contain multiple phenol functional groups (a hydroxyl group bonded to an aromatic ring) [18]. Thousands of polyphenolic compounds are distributed in fruits, vegetables, and beverages such as tea and coffee. These compounds comprise several broad structural classes that each has closely related subclasses of compounds with differing oxidation states and/or substitution patterns [18]. Polyphenolic compounds are divided into four principle structural classes: phenolic acids, stilbenes, lignans, and flavonoids [18]. This section will focus specifically on the phenolic acids and flavonoids due to their dietary prevalence.

1 Phenolic Acids

Phenolic acids are the simplest polyphenols in terms of chemical structure. Phenolic acids are carboxylic acids derived from either benzoic or cinnamic acid skeletons (Fig. 15.2). Predominant dietary phenolic acids and their sources are listed in Table 15.3 [18–21].

2 Flavonoids

Flavonoids are composed of two phenyl rings linked by a propane bridge to form an oxygenated heterocyclic ring with a benzo-γ-pyrone structure, resulting in the

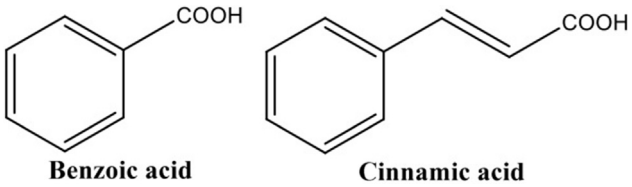


FIGURE 15.2 Structures of benzoic acid and cinnamic acid, the backbones of phenolic acids.

TABLE 15.3 Major Dietary Sources of Water-Soluble and Lipid-Soluble Phytochemicals

Class	Subclass	Representative Compounds	Food Sources
Phenolic acids	Benzoic acids	Gallic acid, protochatechuic acid, hydroxybenzoic acids	Tea, berries
	Cinnamic acids	Chlorogenic acid, coumaric acids, caffeic acids, ferulic acids	Coffee, berries, cherries, apples, cereal grains
Flavonoids	Anthocyanins	Cyanidin, delphinidin, malvidin, pelargondidin, petunidin, peonidin	Grapes, berries
	Flavonols	Kaempferol, myrecitin, quercetin	Apples, onions, leeks, broccoli, tomato
	Flavan-3-ols	Catechins, procyanidins	Tea, grapes, chocolate, apples, berries
	Flavanones	Hesperitin, naringenin, eriodictyol	Citrus and citrus products
	Flavones	Apigenin, luteolin	Green leafy herbs and spices, peppers
	Isoflavones	Daidzein, genestein, glycetin	Soybeans, legumes
Carotenoids	Xanthophylls	Lutein, zeaxanthin, β-cryptoxanthin	Green leafy vegetables, other green vegetables
	Carotenes	α-Carotene, β-carotene, lycopene	Tomatoes, red vegetables, yellow/orange vegetables
Tocochromanols	Tocopherols, tocotrienols	α-, β-, γ-, and δ-Tocopherols and tocotrienols	Nuts, seeds, plant oils, green leafy vegetables, cereal grains
Curcuminoids		Curcumin, desmethoxycurcumin, bis-desmethoxycurcumin	Turmeric, mustard

characteristic C6-C3-C6 flavan skeleton with three rings (Fig. 15.3) [19,22–25]. The A- and B-rings are benzenes, and the C-ring is the central pyran heterocycle. Flavonoids are divided into subclasses based upon the oxidation state, substitution pattern, and functional group composition of the C-ring as well as the nature of the B–C ring linkage [18,19,24,25]. The six major classes of flavonoids are the anthocyanins, flavonols, flavan-3-ols, flavanones, flavones, and isoflavones (Fig. 15.4) [18,19,22,23]. Predominant plant-derived flavonoids and key dietary sources are listed in Table 15.1 [18–21].

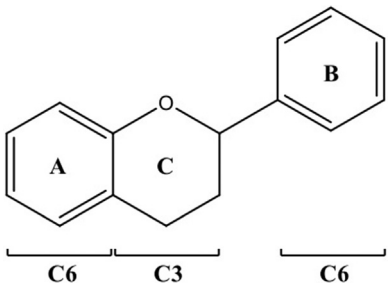


FIGURE 15.3 The C_6–C_3–C_6 three ring flavan skeleton characteristic of flavonoids.

B Bioavailability of Polyphenols

1 Absorption

Ingested polyphenols are present in the gastrointestinal lumen (inner cavity) and technically remain outside of the body until they have been absorbed through the gastrointestinal epithelia. The majority of intact polyphenol absorption occurs in the upper small intestine, with subsequent absorption of major microbial metabolites in the lower intestine. In order for dietary polyphenols to be absorbed in the small intestine, they must first be made bioaccessible. Bioaccessibility is defined as presentation of the compound to the luminal (apical) absorptive surface of intestinal epithelial cells (enterocytes). The bioaccessible fraction (or % bioaccessibility) is the fraction of the consumed phytochemical dose that is extracted from a food during digestion and made accessible and available for absorption by enterocytes.

Several factors determine polyphenol bioaccessibility. First, to be absorbable, polyphenols must be released from molecular interactions with other food components (such as protein, fiber, and lipid) as well as bulk-phase physical interactions with the food matrix. During digestion, polyphenols are released from the food matrix by mechanical action such as chewing and grinding in the

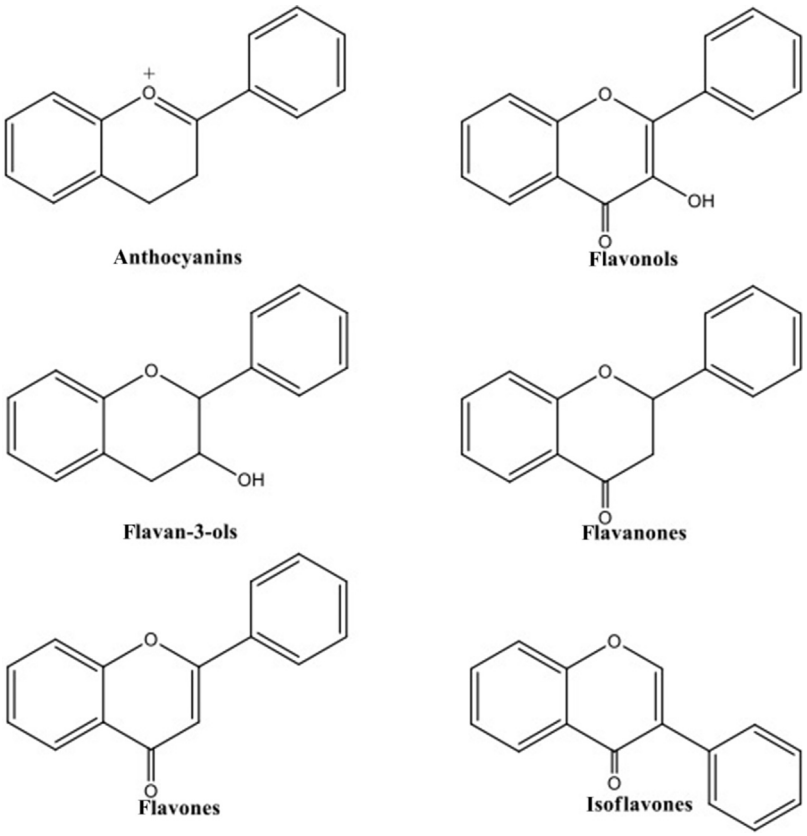


FIGURE 15.4 Basic structural features of the six major subclasses of flavonoids.

oral cavity. Further digestive release continues in the stomach and small intestine due to the action of gastric acid as well as a variety of gastric and intestinal enzymes that hydrolyze lipids, proteins, and carbohydrates. The breakdown of the physical food macrostructure, as well as disruption of molecular interactions between phytochemicals and food components, frees the polyphenols to diffuse into the aqueous milieu of the gastrointestinal tract. Secondly, stability of phenolics in the gastrointestinal tract will affect the concentration available to the intestinal epithelia [26]. Saliva, gastric juice, and intestinal secretions contain a wide array of enzymes designed to degrade food components (pepsin, trypsin, esterases, lipases, amylases, etc.), as well as wide pH variations, that may affect the amount of the ingested dose that remains intact during digestion. Thirdly, polyphenols must be soluble in the bulk aqueous phase, in the gastrointestinal milieu, in order to facilitate the final step: diffusion across the unstirred water layer that protects the absorptive enterocyte surface [27]. Only the fraction of the ingested dose that meets these criteria of stability, release, solubility, and diffusion will be available for absorption (i.e., bioaccessible) (Fig. 15.5). The fractional bioaccessibility varies greatly between polyphenols, depending on the compound, and the nature of the food matrix (macronutrient composition, physical form, etc.) and the whole meal [28].

The portion of the ingested polyphenol dose that reaches the surface of the small intestine is then available for absorption into the enterocytes and subsequent transport to circulation. Identification of specific transport systems for polyphenols remains an active area of research. Intestinal uptake of several polyphenol classes has been attributed to the action of transporters, including monocarboxylic acid transporter, sodium dependent glucose transporter 1, glucose transporter 2 as well as by passive diffusion [29–31] and possible paracellular transport (through spaces between cells). Polyphenols may compete for transport with other polyphenols as well as macronutrients such as glucose, making the process somewhat inefficient [32]. Passive diffusion appears to contribute significantly to absorption of some flavonoids (e.g., isoflavones, flavanones), as the lipophilicity (log P) appears to highly correlate to intestinal permeability and these flavonoids have a high log P value [29]. However, passive transport is a minor contributor to the absorption of others, particularly those which are highly hydroxylated or glycosylated and thus have lower (i.e., more water-soluble) log P values (e.g., flavan-3-ols, anthocyanins, etc.).

Typically, only a small fraction (<20%) of the oral polyphenol dose is absorbed in the small intestine and transported into circulation. One reason for this inefficiency is that enterocytes act as the first barrier against xenobiotics due to their barrier function as well as through the action of efflux transporters. These transporters limit cellular accumulation and transepithelial transport of xenobiotics (and hence, systemic distribution), by inhibiting their initial uptake and then actively removing them from the cell interior and returning them to the lumen, or transporting them to interstitial space, or into the bloodstream. Efflux of polyphenols by enterocytes appears to be facilitated by members of the ATP-binding cassette superfamily of transmembrane transporters, specifically P-glycoprotein (Pgp) and multidrug resistant

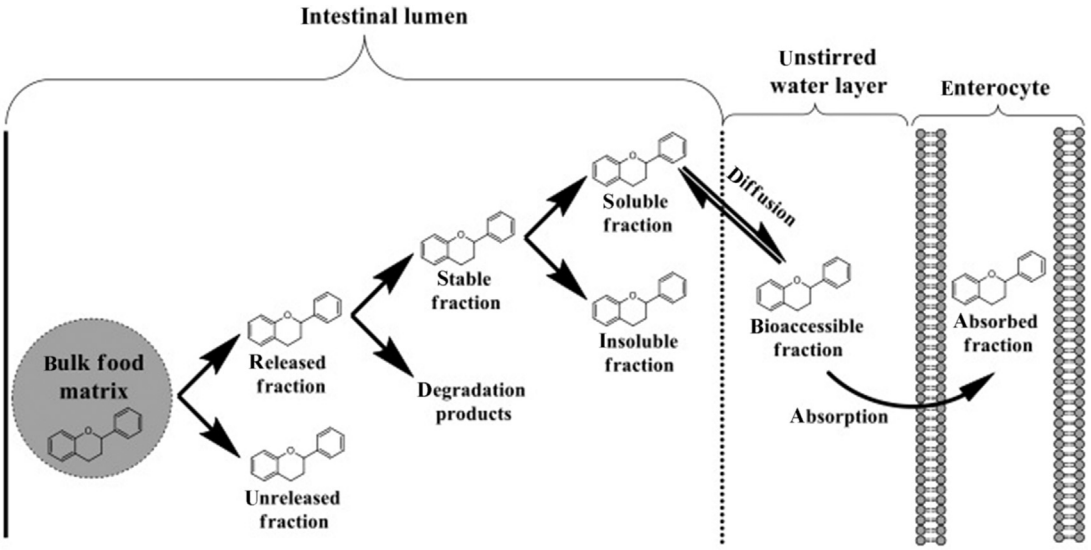


FIGURE 15.5 Digestive and absorptive processes involved in the bioaccessibility of polyphenols.

proteins (MRPs) 1 and 2 [33,34]. This active efflux of xenobiotics has been termed Phase-III metabolism. Pgp and MRP2 are apical transporters that efflux xenobiotics back into the intestinal lumen rather than into the blood stream [30]. MRP1 is expressed on the side of the basal lamina and provides access to the bloodstream via the mesenteric veins on the serosal side of the intestinal epithelia. From the mesenteric veins, these compounds enter the portal vein and are circulated into the liver.

2 Metabolism and Excretion

As xenobiotics, polyphenols are substrates for the body's inducible detoxification system. This system reduces the potential toxicity of foreign compounds by metabolizing them to compounds that are more readily excreted or have reduced biological activity. This detoxification system consists of three primary activities, called phase I, phase II, and phase III metabolism. Phase I metabolism is performed by members of the cytochrome p450 (CYP) superfamily of enzymes, and typically involves hydroxylation. The addition of a hydroxyl group is a functionalization step that renders xenobiotics more reactive to subsequent metabolism, such as phase II. Due to their hydroxylation, polyphenols are not typically substrates for phase-I activation by most CYP phase-I detoxification enzymes [35,36].

Phase II metabolism involves conjugation reactions whereby a hydroxyl group on the xenobiotic is modified by addition of a sulfate, glucuronic acid, or methyl group. Glucuronidation is carried out by uridine diphosphate glucuronyl-transferase (UDPGT), sulfation is carried out by sulfotransferase (SULT) or phenol-sulfotransferase (PST), and O-methylation is carried out by catechol *O*-methyl transferase (COMT) [33,35,37,38]. These reactions can typically occur at any phenol group on the molecule. The products of these reactions can also be substrates for further phase-II metabolism, resulting in the generation of multiple conjugated and/or methylated products. These reactions decrease the potential toxicity of xenobiotics, and facilitate their excretion into bile and/or urine by the liver and kidneys, respectively [35]. Structures of predominant phase-II metabolites of a representative flavonoid are shown in Fig. 15.6. Polyphenol readily undergoes phase-II conjugation in a variety of tissues, particularly the intestinal epithelium, liver, and kidneys.

Polyphenols absorbed from the intestinal lumen by enterocytes are mostly subjected to phase-II detoxification reactions in the cell interior, which is a common site of phase-II metabolism of these compounds in the body.

It is important to note that some phytochemical-rich plants, including foods and herbs, are known to drastically affect the expression and activity of xenobiotic

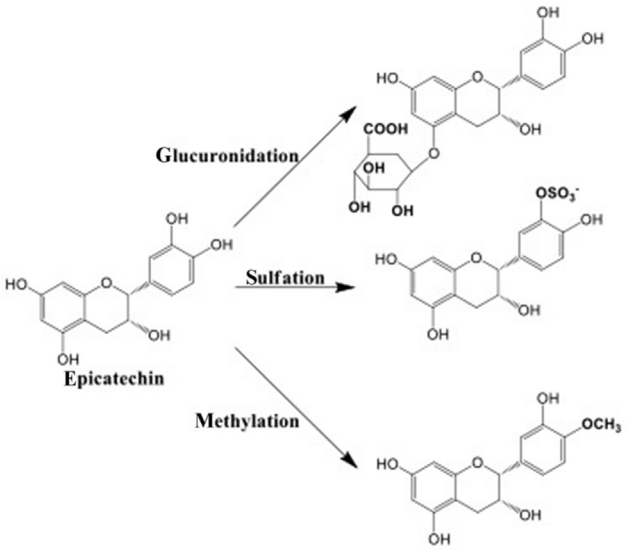


FIGURE 15.6 Structures of selected phase-II metabolites of a representative flavonoid compound [(−)-epicatechin]. Conjugation may occur at a variety of positions on the molecule. Polyphenols may also undergo multiple phase-II modifications on the same molecule.

detoxification systems [39−42]. For example, grapefruit flavanones inhibit detoxification pathways, while phytochemicals from St. John's wort significantly upregulate detoxification pathways. These activities can significantly affect the bioavailability of phytochemicals and pharmaceuticals. Pharmaceutical doses are designed to deliver drugs within their therapeutic window (the range between the minimum effective level and minimum toxic level). Changes in pharmaceutical bioavailability can result in concentrations outside the therapeutic window, often resulting in serious health complication, including death. Upregulation of detoxification can reduce blood levels of pharmaceuticals to below their effective concentration, resulting in loss of efficacy for antirejection drugs for transplant patients, chemotherapeutics for cancer patients, and so on. Conversely, inhibition of detoxification can lead to greater than anticipated blood levels of pharmaceuticals, potentially leading to toxic levels of drugs such as digoxin.

Finally, phase III metabolism involves the efflux of both native xenobiotics and their phase I/II metabolites from enterocytes to lower their intracellular concentration. The phase-II conjugates formed in the enterocytes appear to be efficiently transported into the interstitial space and bloodstream by MRP1 and into the gut lumen by MRP2, but do not appear to be effectively transported by Pgp [33,34,43,44]. Like the native compounds, phase-II conjugates effluxed into the blood stream enter the mesenteric veins and are circulated into the liver by the portal vein prior to systemic circulation.

From intestinal tissues, both native compounds and phase-II metabolites secreted into the bloodstream are passed to the liver through the portal vein. The liver is another key site of extensive xenobiotic metabolism, including phase-II metabolism. Studies have indicated that the activity of COMT is highest in the liver [33,38,45,46]. The liver also possesses strong glucuronidation and sulfation activity from UDPGT and SULT, respectively [33]. The metabolism of dietary xenobiotics in the enterocytes and liver is termed "first pass" metabolism, as this is where absorbed compounds are often first exposed to metabolism prior to entering general circulation.

Circulating forms of polyphenols are largely extracted from the bloodstream by the kidneys and excreted in urine [47]. Glucuronides and sulfates appear to be more readily excreted into the urine than the native forms [48,49]. In addition to urinary elimination, native polyphenols and their O-methylated forms are secreted from the liver into bile, either by first-pass or subsequent metabolism, and appear in feces [50–52]. Excreted compounds may be reabsorbed by the intestines, a phenomenon known as enterohepatic recycling.

The majority of the studies indicate that the total small intestinal absorption of dietary polyphenols is relatively poor, typically accounting for 0.3–43% of the total amount ingested depending on the compound, with most values at the extreme lower end of this range [53]. Therefore, the majority of the ingested dose passes through the small intestine unabsorbed, and reaches the colon. The colon harbors a complex bacterial community comprised of over 500 species, mainly anaerobes of the groups *Bacteriodes*, *Eubacterium*, *Bifidobacterium*, *Fusobacterium*, *Peptostreptococcus*, and *Atopobium* [54–56]. These bacteria reside in both the lumen and the epithelium/mucosa of the colon. The bacterial load of the colon is extremely high, with 10^9–10^{12} cells/g luminal contents, and bacteria can comprise up to 60% of fecal matter by dry weight [57]. Colonic bacteria essentially possess the metabolic potential of an organ, and perform numerous functions critical to the health of the host, including competition versus invasive pathogens, salvage of unabsorbed energy, stimulation of immune function, and control of colonocyte differentiation and proliferation [57–59]. The varied metabolic capacity of colonic bacteria results in extensive fermentation of unabsorbed material. Studies have consistently demonstrated that the colonic bacteria metabolize polyphenols to many simpler metabolites. Similar to native compounds, these metabolites may be absorbed, subjected to xenobiotics metabolism, and excreted. Microbial metabolites may account for many of the reported bioactivities of dietary polyphenols in vivo.

The contribution of colonic fermentation products to overall bioavailability of polyphenols is illustrated well by the flavan-3-ols. Both monomeric flavan-3-ols (catechins) and their oligomeric/polymeric forms (procyanidins) have modest net small intestinal absorption. Typical reports indicate that systemic bioavailability of intact flavan-3-ols is generally poor (<25%), with most studies reporting from 0.1% to 10% of the ingested amount for C, EC, EGC, and EGCG and their phase-II conjugated metabolites [20,45,60–64] and much less for procyanidins (0.3–4%) [65–69]. These data suggest that a large portion of the ingested dose of these compounds is not absorbed in the small intestine but rather reaches the colon and its associated microflora as native compounds or as phase-II metabolites [20,45,70].

Polyphenol glycosides found in many foods and polyphenol conjugates, produced by phase I/II metabolism and apical efflux by enterocytes, are hydrolyzed by colonic microbiota to generate absorbable native polyphenols [71,72].

3 Microbial Metabolism

The result of the poor absorption of many phenolics in the small intestine is that the majority of an oral dose will reach the colon [73,74]. Thus, many of the benefits of dietary phenolics may occur in the gastrointestinal tract [18], with the major point of exposure as the large intestine and its resident microbiota [54–56].

Unabsorbed phenolics that reach the colon are metabolized by the colonic microbiota into several smaller constituents such as simpler flavonoids (from polymerized flavonoids), γ-valerolactones, phenolic acids, and so on [75–77]. For example, predominant microbial metabolites generated from flavan-3-olsols are 5-(*m*, *p*-hydroxyphenyl)-γ-valerolactone, 2-(*m*, *p*-hydroxyphenyl) acetic acid, 2-(*p*-hydroxyphenyl) acetic acid, 2-(*m*-hydroxyphenyl) acetic acid, 3-phenylpropionic acid, 3-(*m*-hydroxyphenyl) propionic acid, 3-(*p*-hydroxyphenyl) propionic acid, 3-(*m*-hydroxyphenyl) valeric acid, ferulic acid, isoferulic acid, vanillic acid, *m*-coumaric acid, *p*-coumaric acid, caffeic acid, gallic acid, and hippuric acid. Structures of a selection of these compounds are shown in Fig. 15.7A. Monomers are metabolized directly into smaller phenolic compounds, while larger compounds must first be broken into smaller degree of polymerization (DP) compounds [76,78]. Furthermore, larger metabolites are then metabolized into smaller and smaller compounds.

Due to the one-way nature of the GI tract and the progressive metabolism of phenolics, gradients of native compounds and their microbial metabolites are likely established along the length of the colon [66] (Fig. 15.7B). However, there are little data regarding distinct profiles present in the lumen of the proximal, mid, and distal colon. Recently, we examined the degradation kinetics of polymerized and monomeric flavanols and

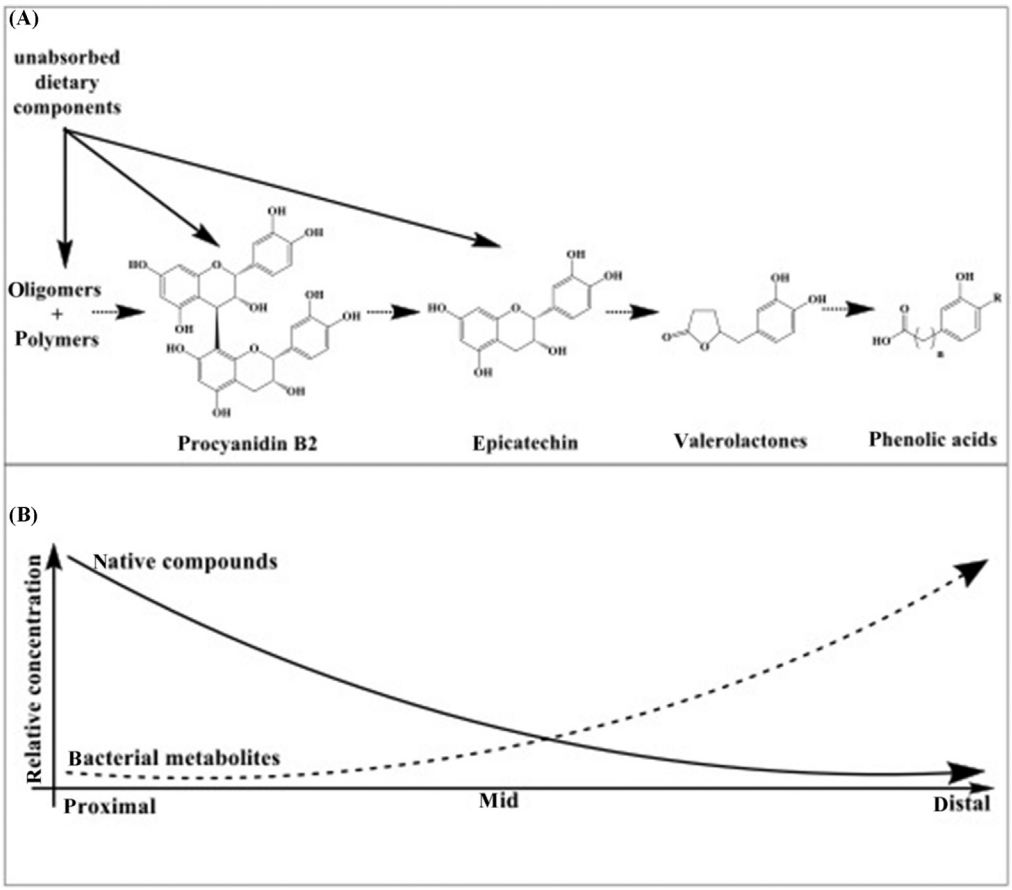


FIGURE 15.7 (A) Highly schematic representation of the colonic metabolism of unabsorbed dietary flavonoids. (B) Representation of the concentration gradients of native flavonoids and their bacterial metabolites that are likely generated along the length of the colon during progressive bacterial metabolism.

formation of their microbial metabolites throughout the length of the lower GI tract in rats [79–81], resulting in several novel findings. The degradation of native species and formation of metabolites appear to occur in all regions of the lower GI tract (cecum, proximal, mid and distal colon). Additionally, all classes of microbial metabolites were formed in each region. This indicates that all regions of the lower GI tract possess the necessary resident bacteria to completely metabolize native flavan-3-ols. Flavan-3-ol metabolism appears to be controlled predominantly by time, as opposed to distinct microbial populations along the lower GI tract. Second, appearance/disappearance kinetics of native compounds and their metabolites in the lower GI tract colon are significantly different: high levels of natives are present early and disappear quickly, while microbial metabolites are present at high levels over longer periods of time.

a Implications of Microbial Metabolism

Flavonoids and other phenolics have been identified as dietary bioactives with potential health-protective activities. Many of these flavonoids have very poor systemic bioavailability. Therefore, circulating concentrations typically represent a small fraction of the ingested dose, whereas the majority reaches the colon unabsorbed. Examples of flavonoids with poor bioavailability include quercetin and procyanidins. Despite poor bioavailability, many of these compounds appear to be effective at preventing or ameliorating chronic disease in vivo, even at low doses. This suggests three possibilities: (1) these flavonoids exert their activities in the gut lumen and epithelium, (2) these flavonoids exert their activities in peripheral tissues even at very low circulating levels, or (3) microbial metabolites of these flavonoids reach circulation and contribute significantly to their observed activities in peripheral tissues. While all three may occur simultaneously, the potential activities of microbial metabolites formed from unabsorbed flavonoids remain poorly understood.

Data suggest that these metabolites are comparatively more bioavailable than their native flavonoid precursors [76,82]. They are absorbed readily from the colon, and in many cases are the predominant circulating compounds

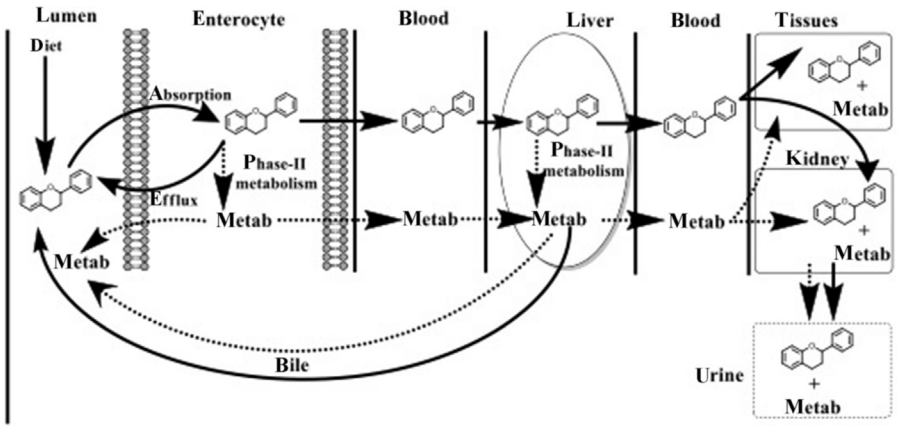


FIGURE 15.8 Schematic representation of processes that affect systemic bioavailability and metabolism of dietary polyphenols. Dotted lines indicate metabolism/metabolites.

derived from of the native flavonoids following flavonoid consumption. While flavonoids have often demonstrated efficacy in models of chronic disease despite poor bioavailability, most studies of flavonoids continue to focus on elucidating the activities, and improving the bioavailability, of the native compounds. The formation and absorption of the microbial metabolites has been extensively characterized, but comparatively little work has been done to characterize the effects of these metabolites in cell or animal models. The potential bioactivities of microbial metabolites have been identified as the potential "missing link" to explain the health benefits of flavonoids with limited bioavailability [83,84]. However, for various reasons, little work has been done yet to make this connection. Understanding these activities may demonstrate a key mechanism by which dietary flavonoids exert their observed effects.

A schematic of the bioavailability of polyphenols, encompassing the factors discussed above, is shown in Fig. 15.8.

b Additional Consideration in Assessment of Phenolic Bioavailability

Polyphenol bioavailability and metabolism is often experimentally assessed after acute oral doses in humans and experimental animals. However, recently there has been increasing awareness that long-term or chronic exposure to polyphenols more closely resembling dietary interventions may alter absorption and metabolism of these compounds, presumably by modifying enterocyte and/or liver physiology, including induction of distinct epithelial barrier function and transport and metabolizing systems. An increase in small intestinal absorption of flavonoids from foods and dietary supplements including green tea and grape seed extract has been documented with as little as 10 days of repeated exposure to oral doses in humans or animals [85,86]. Additional changes in patterns of flavonoid metabolism have been documented, including alteration of glucuronidation and methylation [87]. With inducible transport and metabolizing systems at play, consideration of and controlling for dietary patterns and polyphenol exposure should be included in any assessment of phenolic bioavailability from foods.

It is equally important to consider underlying health conditions that also impact phenolic absorption and metabolizing systems in the gut. For example, both obesity and diabetes are conditions that have been associated with enhanced inflammation [88,89] as well as altered GI motility, GI permeability, hormone secretion, and other factors that may modulate key xenobiotic processes [90–94]. As these at-risk populations are targets for polyphenol-based dietary strategies to promote health, changes in xenobiotic processing due to disease may lead to modification of phenolic absorption and ultimate efficacy. Diabetes is known to alter gastric emptying and motility, as well as expression of phase II xenobiotic enzymes key to generation of biologically relevant glucuronide and sulfate metabolites, including increased UDP-glucuronosyltransferase (UGT1A1) and decreased expression of sulfotransferases (SULT2A1) in experimental models of disease [95,96]. Ongoing research is beginning to define how these changes may potentially impact the design and implementation of long-term dietary strategies and recommendations to promote health in obese or diabetic human populations.

III LIPID-SOLUBLE COMPOUNDS

A Key Classes of Lipid-Soluble Phytochemicals

As described earlier, three main classes of lipid-soluble phytochemicals are predominant in commonly consumed fruits, vegetables, and spices: carotenoids, tocochromanols, and curcuminoids. The carotenoids are bright yellow and orange plant pigments characterized by their 40-carbon structures derived from the 5-carbon precursor

molecule isoprene. As such, carotenoids are long-chain hydrocarbons with highly conjugated double-bond systems, and bilateral or near bilateral symmetry around a central bond [10,97,98]. Carotenoids are further classified based on the presence or absence of end cyclization (e.g., straight-chain lycopene vs cyclized α- and β-carotenes) and the presence or absence of oxygen (strict hydrocarbons such as lycopene and β-carotene are called carotenes, while oxygenated carotenoids such as lutein and zeaxanthin are called xanthophylls; Fig. 15.9) [10,97,98].

The tocochromanols (Fig. 15.9), or vitamin E compounds, are isoprenoids-based amphipathic molecules, with a polar chromane ring head and a nonpolar 16-carbon phytyl tail [99–102]. Tocochromanols have varying methylation patterns in the chromane group, designated as the α−, β−, γ−, δ-tocochromanols. Tocochromanols are comprised of two distinct classes: the tocopherols and the tocotrienols. Tocopherols have a saturated phytyl tail, while tocotrienols have three double bonds in the phytyl tail. Therefore, tocopherols have three chiral centers and can exist as eight stereoisomers (naturally-occurring α-tocopherol is in the all-*R* configuration, while synthetic α-tocopherol is all-*racemic*, or an equal mixture of all eight stereoisomers). Due to the phytyl unsaturation, the tocotrienols have only one chiral center and two stereoisomers (naturally-occurring tocotrienols have the *R* configuration at carbon 2, and *trans*-double bonds at carbons 3′ and 7′).

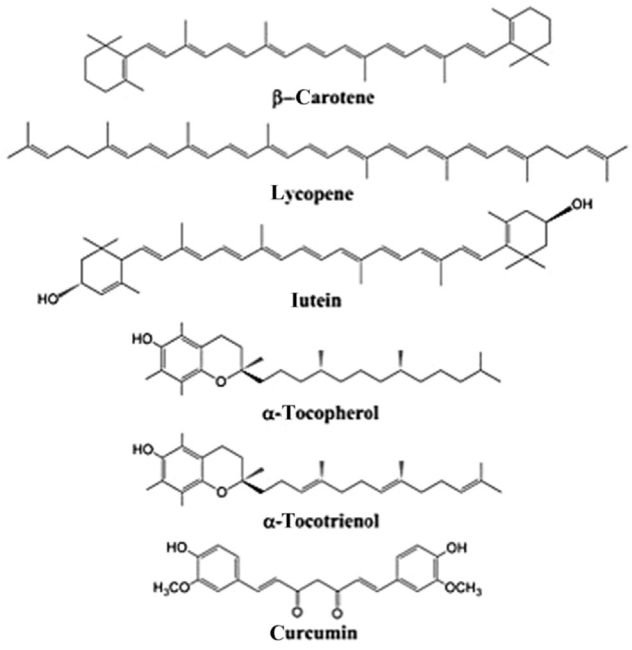


FIGURE 15.9 Structures of lipophilic phytochemicals from various classes: carotene carotenoids (β-carotene and lycopene), a xanthophyll carotenoid (lutein), a tocopherol tocochromanol (α-tocopherol), a tocotrienol tocochromanol (α-tocotrienol), and a curcuminoid (curcumin).

The curcuminoids are diarylheptanoids (two aryl rings linked by a 7-carbon chain). The curcuminoids have varying functional group substitutions on the aryl rings (hydroxy, methoxy, and sulfate and sugar groups), and the aryl rings may be symmetrical or different. Curcuminoids also may have distinct 7-carbon chain patterns (unsaturation, oxo groups, enone groups, 1,3-diols, 1,3-diketones, and cyclization), with all double bonds in the *trans*-configuration. The main curcuminoids in the diet are curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which have the 1,3-diketone chain and differ by the number of aryl rings having a methoxy group (both, one, and neither, respectively) (Fig. 15.9). Although curcuminoids are not isoprenoids, their poor solubility in water and their digestive properties classify them as lipid-soluble phytochemicals.

B Bioavailability of Lipid-Soluble Phytochemicals

As with the water-soluble compounds, only the bioaccessible fraction of any lipid-soluble phytochemical that is ingested will be available in the small intestine for subsequent absorption. However, factors governing the bioaccessibility of lipid-soluble phytochemicals are dependent on the ability of the digestive process to solubilize these highly hydrophobic compounds in the aqueous milieu of the gastrointestinal tract lumen. Solubilization in the gut lumen is highly dependent on coconsumed lipid in the form of triacylglycerols, which, once digested, facilitate formation of bile salt lipid micelles (Fig. 15.10).

1 Bioavailability and Metabolism of Carotenoids

With the established provitamin-A activity of plant-derived carotenoids (α-, β-carotene and β-cryptoxanthin specifically), carotenoid bioavailability has been extensively studied [103]. Digestive release and bioaccessibility represent significant obstacles to carotenoid bioavailability. First, the carotenoids must be released from physical entrapment in the bulk food matrix in order to become bioaccessible [103,104]. For plant tissues, this involves disruption of the plant cell walls and organelles containing the carotenoids [105]. Thus, food processing, such as heating, mechanical breakdown, etc., can significantly increase carotenoid bioavailability simply by increasing the amount able to be released from the food matrix during the normal digestive process [105]. Following digestive release in the stomach and upper small intestine, the hydrophobic components aggregate, due to their poor aqueous solubility, to form crude lipid emulsion droplets in the gastric chyme, which

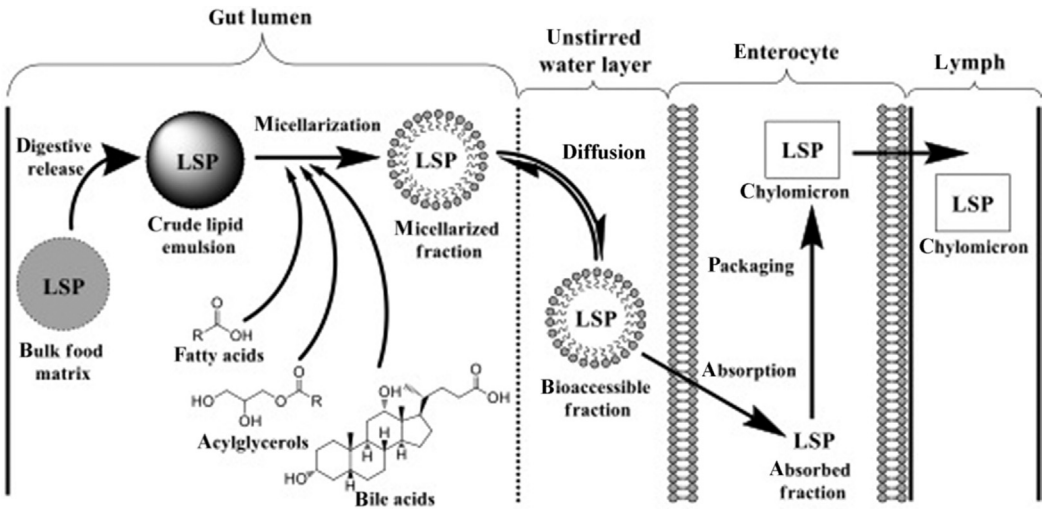


FIGURE 15.10 Schematic representation of processes impacting systemic bioavailability and metabolism of dietary lipophilic compounds.

progressively become smaller as mechanical disruption continues [104]. The carotenoids are then partitioned into mixed micelles in the small intestine. Mixed micelles are heterogeneous aggregates with hydrophilic exteriors and hydrophobic interiors, composed of bile salts, fatty acids, mono- and diacylglycerols, cholesterol, carotenoids, tocochromanols, and so on [98,104,106]. The formation of micelles allows carotenoids to be soluble in the hydrophobic interior while the micelle crosses the unstirred water layer at the intestinal epithelial surface. Carotenoids that escape micellarization are not typically bioaccessible, and remain unabsorbed [103–106]. Due to their relative polarity, xanthophylls (oxygenated carotenoids) are more readily micellarized than apolar carotenes, and are hence generally more bioaccessible [103].

Coconsumption of dietary fat has consistently been shown to improve carotenoid absorption [107–113]. Fat intake stimulates secretion of bile salts and lipases during digestion, and also provides essential components for the formation of mixed micelles (phospholipids, acylglycerols, fatty acids). Fat also assists in extracting carotenoids from the food matrix into the crude lipid emulsion during processing and digestion. Coingestion of 3–5 g fat is thought to provide optimal carotenoid absorption from a meal, and long-chain dietary fatty acids enhance absorption more than short- and medium-chain fatty acids [98,104,106]. However, the threshold value of coconsumed lipid required for efficient carotenoid absorption is still the subject of active investigation.

Upon diffusing across the unstirred water layer, mixed micelles come into contact with the intestinal epithelium, where the micelles are disrupted and the carotenoids are released onto the epithelial surface and then absorbed into the enterocytes by both passive and facilitated diffusion processes. Transporters participating in facilitated carotenoid diffusion include SR-B1, CD36, and NPC1L1 [103,104]. Once inside the enterocytes, carotenoids are transported to the Golgi apparatus with the assistance of fatty acid binding protein (FABP), where they are incorporated into chylomicron particles along with other lipid-soluble compounds and fatty acids and apoprotein B-48 [103]. These chylomicrons are then secreted into the lymphatic system for transport through the thoracic duct and subclavian vein to the liver [104]. During transport, chylomicrons are disrupted by the action of lipoprotein lipase in the vascular compartment, and the resulting chylomicron remnants are taken up by the liver [105]. In the liver, carotenoids are repackaged into very low-density lipoproteins (VLDL) for systemic export. VLDLs are subsequently converted into low-density lipoproteins (LDL) and then high-density lipoproteins (HDL) [103]. The apolar carotenes are believed to reside at the interior of these lipoproteins, while the more polar xanthophylls reside on the exterior [103,105]. During the conversion of VLDL to LDL and HDL, LDL becomes enriched in carotenes while HDL becomes enriched in xanthophylls [103].

The intestinal and hepatic metabolism of provitamin-A carotenoids to retinol as well as conversion of other carotenoids (lycopene, etc.) is the subject of intensive investigation beyond the scope of this chapter [104]. While carotenoids are not generally not metabolized by the classic Phase-I, and -II xenobiotic detoxification pathways, information on generation of apo-carotenoids through cleavage of intact species by BCO1 and BCO2 is yielding insight into factors that impact bioavailability and ultimate disease preventative activities of these plant pigments [114–116].

2 Bioavailability and Metabolism of Tocochromanols

Processes governing bioavailability of tocochromanols appear to be similar to carotenoids [117,118]. Tocochromanols must be released from the bulk food matrix, micellarized, and presented onto the enterocyte surface for absorption, and dietary fat plays a crucial role in this process as for carotenoids [117–122]. The absorption of tocochromanols occurs through both passive and facilitated diffusion, with the potential involvement of SR-BI [118,119,123]. In the enterocytes, tocochromanols are assembled into chylomicrons with the assistance of microsomal triglyceride transport protein, and secreted into the lymph [118,123]. Additionally, some fraction may be exported directly into portal circulation via HDL [118,119,123]. The liver absorbs chylomicron remnants, and tocochromanols are then repacked into VLDL for systemic distribution [102,119]. The majority of circulating tocochromanols reside in LDL and HDL, from which they are quickly deposited into tissues with the assistance of lipoprotein lipase [119]. In cells, tocochromanols are incorporated into cell membranes as antioxidants, and are not generally metabolized by the xenobiotic detoxification systems.

3 Bioavailability and Metabolism of Curcuminoids

Less is known regarding the bioavailability of curcuminoids compared to carotenoids and tocochromanols. Due to their observed effects in vitro via a wide assortment of mechanisms, there is great interest in their potential preventive and therapeutic activities, including: antimicrobial, antioxidant, antiinflammatory, anticarcinogenic, antithrombotic, neuroprotective, antirheumatic, and hypoglycemic activities [16,17,124–126].

Curcuminoids are hydrophobic polyphenols, with poor solubility in water [17,124,127], leading to poor intestinal solubilization and extremely low gut absorption and bioavailability relative to other polyphenols and also the lipid-soluble phytochemicals. The majority of an oral curcumin dose is not absorbed, but is excreted intact in feces (which also indicates limited metabolism by colonic microbiota) [17,124,125]. In many cases, native curcumin and its metabolites are present at levels near or below the analytical limit of detection in human blood fractions or urine following oral ingestion of gram quantities of curcuminoids [17,124]. The small fraction of curcumin that is absorbed is rapidly biotransformed by reduction reactions (to tetrahydro- and hexahydrocurcumin derivatives), as well as conjugated to sulfates and glucuronides, in the intestinal mucosa, liver, and kidneys [124,127–129]. The low levels of curcumin detected in blood and urine are almost exclusively conjugates of curcumin or reduced curcumin derivatives, and the half-lives of these compounds are exceedingly short due to rapid clearance [124,127–129].

Poor absorption, extensive biotransformation, and rapid elimination pose a major hurdle in exploiting the bioactivities of orally administered curcuminoids [16,17,124,127,129]. Studies of curcumin pharmacokinetics and pharmacodynamics typically rely on doses of gram per kilogram body weight in animals and grams in humans, which are much higher than doses typically employed for other phytochemicals [124,130]. Although curcumin is generally thought to be safe at high doses for short-term use, and has "generally recognized as safe" (GRAS) status in the United States, concerns have arisen over its long-term safety [17,124,127,129,130]. This is of particular importance for long-term use of curcumin as a preventive agent in healthy populations, where toxicity is less acceptable than in therapeutic uses for existing disease.

Several delivery strategies have been developed to improve the bioavailability and efficacy of curcuminoids in vivo. These include (1) coadministration with adjuvants to inhibit metabolism, (2) formulation of nanoparticles targeting delivery of curcumin to specific regions of the GI tract, (3) incorporation of curcumin into micelles, phospholipid complexes, and liposomes to improve diffusion across the unstirred water layer, (4) development of synthetic curcumin analogues with improved solubility, (5) heat-assisted solubilization, (6) synthesis of curcumin prodrugs which yield curcumin upon hydrolysis, and (7) formulation of curcumin metal chelation complexes to improve solubility [16,17,124,127,129–132]. Particularly noteworthy is the use of the adjuvant piperine, which is an alkaloid from black pepper. Coadministration of milligram quantities of piperine with curcumin has been shown to increase the bioavailability of curcumin by up to 2000% [16,124,129,130]. Piperine inhibits phase-II biotransformation and clearance of curcumin, increasing the persistence of native curcumin in circulation [16,124,129,130]. Piperine may also increase gut permeability [133,134]. Concern has recently arisen regarding this adjuvant, as reduced biotransformation of other drugs may result in toxicity due to abnormal accumulation [130].

Curcumin poses a particular challenge for correlating bioavailability to efficacy, as (1) curcumin appears to exert some efficacy even when no native or conjugated curcumin is detectable in blood, and (2) curcumin exerts activities distant from the GI tract, even though this is the primary site of delivery and accumulation. Therefore, the effective dose, active moiety, and mechanisms of action of curcumin remain to be fully characterized in vivo [17,125].

IV SUMMARY

Numerous bioactive phytochemicals are found in commonly consumed plant foods, including fruits, vegetables, beverages, and spices. Their widespread presence in the diet and apparent low toxicity suggest that phytochemicals have the potential to impact human health and disease risk on the population level. Considering this potential benefit, interest in factors affecting their bioavailability from common dietary sources has grown. When considering bioavailability, dietary phytochemicals can be divided into two distinct classes: water-soluble (phenolics and polyphenols) and lipid-soluble (carotenoids, tocochromanols, and curcuminoids). Significant efforts have been placed on the identification of food factors (macro- and microcomposition, physical form, phytochemical concentration, etc.) and in vivo biological factors (digestion, absorption, metabolism, distribution, and excretion processes) which impact bioavailability in humans and experimental animal models. Such knowledge is critical to improve the bioavailability of health-promoting phytochemicals, and to better understand the mechanisms by which they exert their biological activities at target tissues.

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Chapter 16

Antioxidants in Health and Disease

Rebecca M. Seifried[1], **Earl Harrison**[2] **and Harold E. Seifried**[3]

[1]Walter Reed National Military Medical Center, Bethesda, MD, United States, [2]Ohio State University, Columbus, OH, United States, [3]National Cancer Institute, Rockville, MD, United States

I INTRODUCTION

Antioxidants are a loosely characterized group of compounds that are defined by their general ability to decrease or delay oxidative reactions. Dietary antioxidants are recognized to have the ability to inhibit the formation of both reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can adversely affect normal cellular processes and physiological functions [1–4]. Under normal conditions, the balance between production and elimination of free radicals is maintained by endogenous enzymes (e.g., glutathione peroxidases, catalase and superoxide dismutases, thioredoxin reductase, and heme oxygenase) and a host of nonenzymatic components (some metals, glutathione, thiols, certain vitamins, and phytochemicals including flavonoids and other phenolics). Many of these components can be influenced by dietary patterns and behaviors [1–4]. Table 16.1 includes a number of these antioxidants with their respective structures and properties.

The range of chemical classes within the antioxidant domain is tremendous and is part of the reason why grouping of compounds by their ability to interact with reactive species (ROS and RNS) can lead to oversimplification and generalization. Hence, descriptors such as "conundrum" and "double-edged sword" are often used to characterize the relationship between antioxidants and health. Part of the controversy stems from the innate properties of ROS as a class of biologically active compounds that can influence both disease prevention and disease promotion. Although the generation of ROS had been viewed as primarily, or solely, detrimental to health, advances in research have demonstrated that ROS can have crucial roles in normal physiological processes, including functioning as growth factors, influencing immunocompetence, and initiating apoptosis in damaged cells. Despite these beneficial functions of ROS, abnormal production or nonhomeostatic regulation of ROS is linked to the development of many chronic diseases and associated conditions, including cancer and cardiovascular disease (CVD), as well as a number of neurological and metabolic diseases.

In consideration of these facts, antioxidants have been highlighted as promising preventative and or therapeutic agents for several of these diseases. Much of this optimism is based on the frequently observed case–control association between diets high in antioxidant-rich fruits and vegetables (and presumably antioxidant exposures) and decreased disease risk. Although there is evidence that antioxidants may offer health benefits in populations that are at increased risk because of environmental or medical conditions, there are many inconsistencies in the literature. Some of this inconsistency stems from different study end points (i.e., mortality, cancer, or CVD occurrence and individual study results vs meta-analysis of several "related" studies) and study design (prospective vs retrospective, the use of food frequency data vs controlled dietary or purified agent clinical trials, healthy population epidemiological vs disease intervention studies, and so on). The effect of the vehicle that contains the antioxidant also introduced variability because the bioavailability in a food matrix can be quite different from that in a pure compound, and synergistic interactions are likely within the whole food because there are several antioxidants present in most foods. Because evidence does exist that several antioxidants at "normal" exposure levels can regulate signal transduction and, thus, regulate proliferation and the immune response, both normal physiological processes and mechanisms other than their antioxidant properties may be functioning. Overall, the physiological or pharmacological importance of antioxidants as regulators of radicals as a possible means for promoting health continues to receive widespread scientific attention and debate in the literature and therefore

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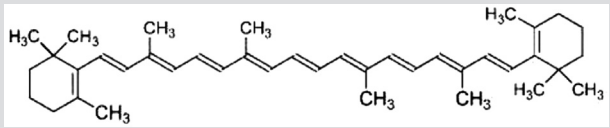
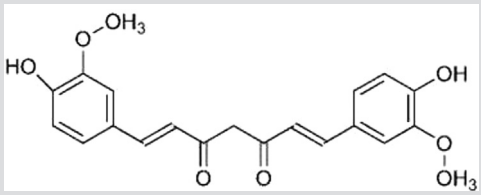
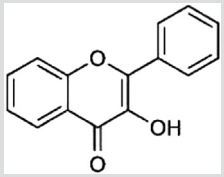
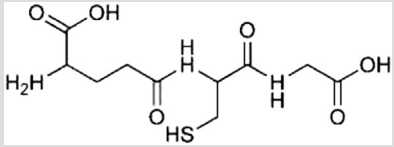
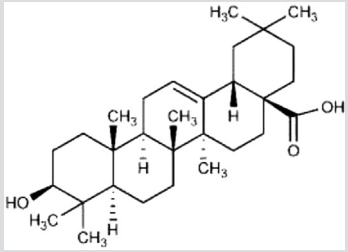
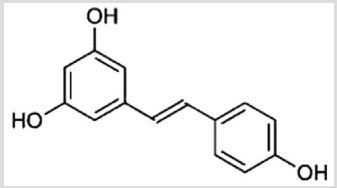
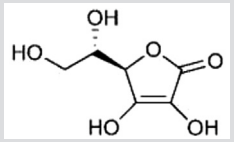
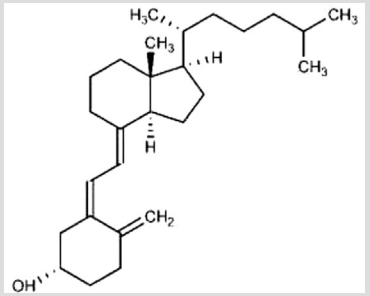
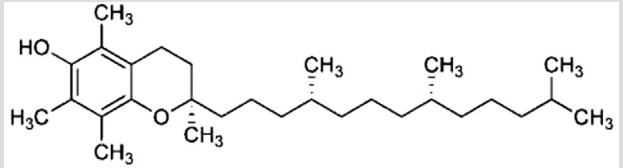
TABLE 16.1 Some Antioxidants and Their Biological Activity

Antioxidant	Structure	Detected or Effective Level	Cancer Activity	CVD Activity	Hormonal Response	Deficiency Symptoms
Carotenoids	β-Carotene 	5–50 μg/dL	Induces connection–differentiation, immune function	Food sources lower CVD	No	Only vitamin A—xeroderma, night vision
Curcumin		>2 μmol/L at 8 g/day	Antiinflammatory; increases apoptosis, interferes with chemotherapeutics and cell signaling; interferes with Phase I enzymes, stimulates Phase II	unknown	No	No
Flavonoids	Genistein 	1–10 μmol/L	Modulates cell signaling, inhibits angiogenesis, increases apoptosis, much data from cell studies	Food sources lower CVD, minimal cholesterol lowering	Some are estrogenic, some anti, some both (does related)	No
Glutathione			Substrate of GSH glutathione peroxidase reduction of H_2O_2 and lipid hydroperoxides and substrate for Phase II carcinogen metabolism	unknown	No	unknown
Oleanic acid			Inhibits proliferation angiogenesis, metastasis; induces apoptosis and cell differentiation; antiinflammatory	Antiatherosclerotic, hypolioidemic	No	No

Resveratrol		Mostly in cell culture	Inhibits proliferation angiogenesis, induces apoptosis and cell cycle arrest in cancer cells in culture	Inhibits platelet aggregation cell adhesion in culture	Both estrogen (E2) agonist without E2 and antagonist with E2	No
Selenium	Se	45−80 μg/L 40−100 μg/day	Increases glutathione peroxidase, stimulates immunity	Weakly cardioprotective	No	Muscle wasting, cardiomyopathy
Vitamin C		Saturated blood levels at 400 mg per day ~ 70 μmol/L	Reduces lung, breast, oral, upper GI, gastric, colorectal CA with oral C; IV doses increase survival	Mixed results on CVD reduction, more + than −, lowers blood pressure, coronary artery dilation	No	Scurvy, impaired collagen synthesis
Vitamin D			Induces differentiation, lowers proliferation	Lowers blood pressure	No	Rickets, seizures, osteomalacia
Vitamin E		20 μmol/L	Affects cell signaling, boosts immunity, inflammation; minimal CA effect	Platelet aggregation, lower MI, carotid atherosclerosis	No	27 to 40 + % of population below 20 μmol/L

continues to be at the forefront of discussion for disease preventative and therapeutic strategies (http://www.cancer.gov/about-cancer/causes-prevention/risk/diet/antioxidants-fact-sheet and http://preventcancer.aicr.org/site/News2?page = NewsArticle&id = 10655&news_iv_ctrl = 0&abbr = pr_hf_).

A Antioxidant Usage and Measures of Oxidative Stress

Both public interest and the scientific literature related to the purported health benefits of antioxidants [5–9] are often thought to parallel the increased use of antioxidant supplements by the U.S. adult population. However, meta-analysis of the available studies may not strongly support such a positive view [6,7]. Data from the National Health and Nutrition Examination Surveys (NHANES), first conducted in 1971, indicated an overall prevalence of dietary supplement usage of 23% in NHANES I; prevalence rates increased to 35% in NHANES II and 40% in NHANES III, with women's usage approximately 12% higher than that of men [10–12]. However, the total male intake was somewhat higher than that of females of all ages [13] (see also https://wwwn.cdc.gov/Nchs/Nhanes/2009-2010/DS1IDS_F.htm [14]). Based on 1999–2000 NHANES III data, approximately 33% take multivitamins and more than 12% use vitamin E or C supplements [5]. The increased interest in the health effects of vitamins in general and specifically the class of "antioxidants" as protecting agents was sparked by a series of articles beginning in the 1970s. In the decades since, an expanded knowledge base in the nutritional sciences about the potential molecular targets and interactions that may account for the health benefits of antioxidants has surfaced from a wide range of preclinical, clinical, and population studies [9,10,15] but there have also been cautionary reports and analyses concerning over-usage and potential harm [6,7].

The major cellular targets of ROS include membrane lipids, proteins, nucleic acids, and carbohydrates. Several biomarkers for oxidative damage and antioxidant defense have been introduced as potential indicators of alterations in normal homeostatic mechanisms. Basically, oxidative stress biomarkers can be separated into those that (1) reflect modified molecules caused by ROS and (2) reflect shifts in biological measures of low-molecular-weight compounds or the induction of enzymes (Table 16.2). The ability to monitor these biomarkers at multiple time points in blood, urine, ductal lavage, and so on allows for repeated measurement and greater sensitivity in detecting and understanding stress status, which is not always possible for multiple reasons, including ethical and patient compliance concerns with more invasive procedures. Unfortunately, it is not simple to link these biomarkers to a specific clinical outcome. However, the field continues to expand and mature. The uncertainty about normal and abnormal values, appropriate target tissues, and the unsubstantiated relationship of a change in a biomarker to a specific phenotypic response also raises significant concerns about many of these measures. Eventually, an "oxidative stress profile" that incorporates changes in multiple biomarkers could be useful in establishing who may benefit or who may potentially be placed at risk by the use of antioxidant supplements or through specific antioxidant-rich dietary interventions.

Two main types of methods have been used to evaluate the antioxidant properties of foods; these assays are based on hydrogen atom transfer (HAT) reactions or on

TABLE 16.2 Some Biomarkers of Oxidative Damage

Total Antioxidant Potential	Total Radical Trapping Antioxidant
Lipid peroxidation	Malondialdehyde-lysine, 4-hydroxy-2-nonenallysine, acrolein-lysine, F2-isoprostane, thiobarbituric acid reactive substances
DNA oxidation	8-Hydroxy-2′-deoxyguanosine
Glyco-oxidation	Carboxymethyl-lysine, pentosidine, argpyrimidine, methylglyoxal
Nitro-oxidation	Nitrotyrosine, nitrite to nitrate
Protein oxidation	*o*,*o*′-Dityrosine, *ortho*-tyrosine, bilirubin oxidative metabolites, oxidized glutathione
Enzyme activities	Superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase, thioredoxin reductase, heme oxygenase
Protein concentrations	Albumin, ferritin, transferrin, lactoferrin, ceruloplasmin, thioredoxin
Concentrations of low-molecular-weight molecules	Bilirubin, tocopherols, carotenoids, ubiquinol/ubiquinone, ascorbate, glutathione, cysteine, urate, selenium

electron transfer (ET). Most HAT-based assays use a competitive reaction scheme in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays include inhibition of induced low-density lipoprotein autoxidation, oxygen radical absorbance capacity, total radical trapping antioxidant parameter, and crocin bleaching assays. The ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant and corresponding change in color when reduced. ET-based assays include the total phenols assay by Folin−Ciocalteu reagent, Trolox equivalence antioxidant capacity, ferric ion reducing antioxidant power, "total antioxidant potential" assay using a Cu(II) complex as an oxidant, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), the oxygen radical absorbance capacity [16,17]. Although each of the methods has value for a relative comparison across a variety of food items, it remains unclear whether the measures truly reflect their physiological value after consumption because they do not measure true biomarkers of physiological activity. In addition, the lack of detailed information about multiple exposures and with variable durations makes the interpretation of existing studies, with sometimes subtle changes in fluids and cells in humans, extremely challenging. The merits of these analyses may also be questioned because possible postprandial or diurnal variations that are not directly related to the intake of dietary antioxidants per se are not considered. Finally, plasma antioxidant capacity may be significantly affected by nonantioxidant dietary constituents, which influence uptake, tissue mobilization, or metabolism of endogenous or exogenous antioxidants [2,15,16,18−20].

The potential beneficial health effects of fruits and vegetables have been attributed, at least in part, to their antioxidant content. Significant and generally transient increases in plasma total antioxidant capacity are frequently observed following the human consumption of flavonoid-rich foods [18]. These flavonoids or possibly other bioactive food components function by modifying oxidative stress has been challenged by observations that typical intakes only result in minimal shifts in circulating biomarkers in plasma, and that extensive metabolism of the agents likely diminishes their in vivo antioxidant capacity. Lotito and Frei [18] concluded that the large increase in plasma total antioxidant capacity observed after the consumption of flavonoid-rich foods is not caused by the flavonoids per se but is likely the consequence of increased uric acid levels. However, work on the metabolism of the glycoside or aglycone forms of the flavonoids has shown that metabolic products, including products of intestinal and hepatic metabolism, can have very different properties than the parent compounds, calling into question the extrapolation of observed in vitro antiinflammatory and antioxidant effects to the in vivo situation [19], whether the appropriate agents and studies are being investigated [19−21] and the accuracy of dietary reporting methods in current use.

The possible usefulness of antioxidants in disease prevention, particularly for cardiovascular, ocular, and neurological diseases as well as cancer, stems from a number of epidemiological findings and a number of follow-up intervention studies. However, a substantive compendium of negative cardiovascular and other effects of antioxidant use, especially relating to dietary supplements, has also emerged, and will be discussed subsequently. A resulting concern is the potentially deleterious effects of antioxidant supplements on normal ROS levels because precise control of ROS levels is needed to allow normal cell function or to promote apoptotic cell death of aged, precancerous, or transformed cells [21]. Conflicting findings on risks and benefits have led to the careful reviews of antioxidant efficacy, such as evidence reports from the Agency for Healthcare Research and Quality (AHRQ) on vitamin C, vitamin E, and coenzyme Q10 [22,23] and a Cochrane review of vitamins A, C, E, and β-carotene [20,21]. The AHRQ reports included broad, systematic searches of the literature; the Cochrane report focused on mortality. The report on prevention and treatment of CVD [22,23] concluded that the evidence did not support a positive benefit of vitamin E supplementation for cardiovascular events; neither did it support significant potential for harm. Conclusions about vitamin C and coenzyme Q were mixed. The review of the cancer literature [23] did not support the hypothesis that supplements of vitamins C or E or coenzyme Q10 generally help prevent or treat cancer. In this context it is important to always consider that isolated findings of benefit require confirmation. Taylor and Greenwald [24] reviewed a number of completed nutritional cancer prevention trials, helping to fill out the background picture on this question. The AHRQ standing has also been reported for specifically vitamin E and β-carotene as "there is no net benefit of supplementation with vitamin E or β-carotene for the prevention of cardiovascular disease or cancer" (Section 2, Recommendations for Adults (continued). Agency for Healthcare Research and Quality, Rockville, MD, June 2014. http://www.ahrq.gov/professionals/clinicians-providers/guidelines-recommendations/guide/section2d.html).

This collection of potential positive and negative antioxidant effects on ROS deserves further examination because of the molecular evidence for the multiple roles of ROS in the development and progression of cancer, CVD, and other diseases [2,15]. This increased attention is timely because there have been studies [2] that link some nutritional antioxidants with increased mortality from cancer and CVD, as well as some clinical studies

that do not support the cancer prevention efficacy of some antioxidants. A review [25] indicated there are a number of European antioxidant and cancer clinical and epidemiological studies under way, so the subject will continue to be closely scrutinized and debated as new information becomes available.

B ROS and Normal Physiology

ROS typically arises as by-products of cellular metabolism and ionizing radiation, usually reflected in the formation of the following four species: superoxide anion (O_2^-) hydrogen peroxide (H_2O_2), hydroxyl radical (OH*), and singlet oxygen ($^1O_2$). Although H_2O_2 and $^1O_2$ are not free radicals per se, these species often initiate and promote oxidation by their ability to react directly with electron-rich organic species. The reactivity of O_2^- or H_2O_2 with other molecules is not appreciable, but the presence of trace amounts of transition metals fosters their conversion to OH via the Fenton or Haber–Weiss reactions. ROS formation is a natural consequence of aerobic metabolism and is integral for maintaining tissue oxygen homeostasis [20,26].

Oxygen homeostasis—the balance between constitutive oxidants and antioxidants—is maintained through a natural series of reduction-oxidation (redox) reactions involving the transfer of electrons between two chemical species: compounds that lose electrons (oxidized) and those that gain electrons (reduced). When oxygen homeostasis is not maintained, the cellular environment becomes oxidatively stressed. Approximately 1–3% of oxygen consumed by the body is converted into ROS [27]. Three of the major ROS—superoxide radical O_2^-, hydrogen peroxide, and hydroxyl radical OH*—are normal metabolic by-products that are generated continuously by the mitochondria in growing cells [26,28,29]. Other significant intracellular sources of ROS include microsomal cytochrome P450 enzymes, flavoprotein oxidases, and peroxisomal enzymes involved in fatty acid metabolism [26]. The potentially damaging oxidative stress can be caused by excess ROS, which are kept in check by endogenous cellular antioxidant mechanisms. Oxidative stress-related enzymes include superoxide dismutases for eliminating the superoxide radical, as well as catalase and glutathione peroxidases for removing hydrogen peroxide and organic peroxides [26,28]. Polymorphisms have been observed in these enzymes, which can affect an individual's capacity to respond to changes in ROS; this is discussed in more detail later.

Transient fluctuations of ROS levels can influence activity of signal transduction pathways leading to cell proliferation or to apoptosis or necrosis, depending on the dosage and duration of ROS changes and also on cell type. Typically, low levels of ROS can be mitogenic, whereas medium (normal homeostatic) levels lead to temporary or permanent growth arrest (replicative senescence), and elevated levels usually result in cell death either by apoptosis or by necrosis [29–31]. Although necrosis and apoptosis may be viewed as negative events in terms of cell loss, these processes also have positive roles in the downregulation of immune responses [3] and the elimination of transformed cells ("tumor suppression" via apoptosis).

C ROS in Disease Conditions

Imbalanced ROS homeostasis has been linked to an increased risk of several diseases such as cancer, CVD, atherosclerosis, diabetes, and neurodegenerative conditions including Alzheimer's disease [32–38]. Understanding the molecular effects of ROS on these different disease processes should assist in unraveling the varied and sometimes contradictory evidence about these diseases and assist in evaluating the importance and safety of antioxidants.

1 Cancer

Cancer is a number of somewhat distinct diseases that can be characterized on the basis of uncontrolled cellular growth resulting from a series of altered sets of genetic and epigenetic manifestations. Hanahan and Weinberg [33] indicated the "hallmark capabilities" necessary for tumorigenesis: (1) self-sufficiency in growth signals, (2) insensitivity to antigrowth signals, (3) evasion of apoptosis, (4) limitless potential for replication, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. Excessive ROS and RNS are involved in all these processes and thus contribute to cancer progression either positively by promoting cell division or negatively by stimulating apoptosis and slowing the growth. Belief in the protective effects of antioxidant supplements has led to their widespread use by cancer patients [34].

The expanded use of nutritional supplements is not limited to the United States. Many other countries are reporting increased use of various alternative and complementary approaches and strategies [35]. A greater understanding of both the negative and the positive consequences of ROS and antioxidants in the etiology and progression of carcinogenesis is crucial to making clear advances in cancer prevention and treatment. Currently, the two faces (benefit/risk) of ROS/RNS in malignant diseases make it difficult to present a true, clear, and concise message to consumers.

2 Cardiovascular Disease

CVD, encompassing atherosclerosis and its associated vascular disorders, is the leading cause of mortality in developed countries [36,37]. Atherosclerosis has three characteristics: inflammation, disturbed blood flow and abnormal shear stress, and arterial wall remodeling. Oxidative stress is associated with impaired arterial elasticity

and excessive ROS can contribute to the structural remodeling through smooth muscle proliferation and enhanced inflammation [38–40]. Other vascular insults such as those associated with cigarette smoking, diabetes mellitus, hypertension, autoimmune disease, and hyperlipidemia can trigger an inflammatory response in blood vessels. Chronic low-grade inflammation is generally accepted to accompany atherogenesis [41,42]. This inflammatory state, which has been linked in part to ROS mediation, can result in damage to smooth muscle and vascular endothelial cells. This in turn leads to a dysfunctional endothelium characterized by pathological alterations in the endothelial cell's anticoagulant, antiinflammatory, and vascular-relaxation properties, which can promote the recruitment of monocytes, macrophages, growth factors, and cellular hypertrophy. All of these factors can contribute to atherosclerotic plaque formation. In summary, increased ROS and related oxidative stress helps drive many of these processes involved in the development of CVD if left unchecked.

3 Neurodegenerative Disease

Neurodegenerative diseases and disorders (NDDs), including dementias such as Alzheimer's disease, are a rising public health problem due to the aging of populations and lack of effective treatments or a cure. As for CVD, there is a growing body of knowledge that suggests that the pathophysiology of NDDs involves mechanisms of oxidative stress and inflammation. There is also an accumulating literature on the positive effects of dietary antioxidants on delaying the progression or slowing the decline of moderate cognitive impairment (MCI), a precursor of frank dementia. Thus, a number of reviews have highlighted the effects of several classes of dietary antioxidants on both biomarkers and functional indicators of cognitive decline [43,44]. The evidence includes results from both prospective and retrospective epidemiological studies and from randomized controlled trials (RCTs). As with nutritional interventions in other diseases, there are reports of positive effects as well as those showing no benefit. However, the current state of knowledge suggests that it would be prudent to continue to investigate the mechanisms of the effects of dietary antioxidants on neurodegenerative processes. Further trials are necessary to more clearly define populations of individuals suffering from or at risk for NDDs who may benefit from consumption of antioxidant-rich diets or supplements.

II ANTIOXIDANTS IN DISEASE ETIOLOGY, PREVENTION, AND TREATMENT

Several studies have reported that diets high in fruits and vegetables can be associated with a decreased risk of CVD and cancer. This has been frequently attributed to high levels of antioxidants present in these foods. Other studies, however, do not provide clear and unequivocal support for this assumption. For example, data from historical food frequency questionnaires collected during the cohort Nurses' Health and Health Professionals Follow-Up Studies indicated that cancer incidence was not influenced by increased fruit or vegetable consumption; however, modest reductions in CVD were detected (Table 16.3) [45]. Similarly, studies of dietary supplements and individual antioxidants are not consistent; observed effects have ranged from benefit to possible harm [46,47] but could be affected by imprecise estimates of dietary intake. A number of other factors may contribute to these contradictory findings, including participant baseline health and ROS levels, exposure to environmental carcinogens, and genetic differences in ROS metabolism [48]. Some argue that higher doses of some antioxidants have a pro-oxidant effect [2], and there is a high probability that pharmacokinetics, including absorption, distribution, metabolism, and excretion of a simple ingested supplement, is quite different from the complex matrix in fruits, vegetables, and even complex meals, which contains many different types of antioxidants as well as other macro- and micronutrients and other phytochemicals.

Because many examples of J- and U-shaped dose–response curves have been reported among vitamins, other nutrients, and food additives, a critical question for cancer patients in active therapy should be the following: What is the potential interaction of ROS and antioxidants with chemotherapy and radiation therapy? The interaction occurs sometimes in a positive manner, enhancing the efficacy of the treatment, but also sometimes negatively, interfering with the agent or treatment. Similarly, individual antioxidants can inhibit or stimulate tumor cell growth or survival depending on the tumor, the antioxidant, and the oxidative status. This means that there is no simple answer regarding the overall effectiveness of antioxidants, and each must be considered on a case-by-case basis. The following sections provide the results of some major clinical and epidemiological trials on the antioxidant effects on cancer and observed side effects, particularly on CVD, focusing on the antioxidants selenium, vitamin E, vitamin C, and β-carotene, all of which are readily available in the marketplace as dietary supplements. Key study findings and some experimental details are presented in Table 16.3.

A Antioxidants and Cancer

The potential benefits of the dietary antioxidants selenium, vitamin E, vitamin C, and β-carotene in numerous observational and clinical trials have been examined for several years [7,49]. Since the 1990s, however, evidence has emerged indicating that some antioxidants, in certain

TABLE 16.3 Human Intervention Studies on Antioxidants

Study and Publication Date	Study Details, Size, and Duration	Intervention Details	Study Results
Linxian Study—China (1993) [52]	29,584 men (5 years)	βC (15 mg), vitamin E (30 mg), and selenium (50 mg) daily	Protective effects: cancer: −13%; mortality: −9%
ATBC Study—Finland (1994) [67,69,70,77−81,102]	29,133 male smokers (5−8 years) with up to 19-year follow-up	βC (20 mg) ± vitamin E (50 mg) daily	Lung cancer: +18% but mortality mixed Prostate cancer: −32% Colorectal cancer: −22% No decrease in CVD or angina Slight excess hemorrhagic stroke and βC-related mortality (+7%), but decreased with vitamin E
CARET Study—United States (1996) [47,68,71]	18,314 male smokers or asbestos exposed (4 years + 6-year follow-up)	βC (30 mg) ± vitamin A (25,000 IU) daily	Lung cancer: +28%; mortality: +17%; baseline levels after 10 years
CHAOS—United Kingdom (1996) [110]	2002 atherosclerosis patients, ∼80% males (510 days)	535 or 263 mg vitamin E daily	Decreased nonfatal MI (77%)
PHS—United States (1996) [54,56,128]	22,071 physicians (12 years) (11% smokers, 39% former smokers)	50 mg βC every other day	No significant overall effect on cancer or CVD; decreased prostate cancer −32%
NPC Trial—United States (1996) [86−88]	1312 men and women with dermal basal or squamous cell carcinoma (4.5 years + 6.4-year follow-up)	200 mg selenium daily	No effect on skin cancer; lung (−46%); colorectal (−58%); prostate (−63%); mortality (−50%)
WHS—United States (1999) [55,92,108]	39,876 female 45 + year-old health professionals (2.1 years + 10-year follow-up) (13% smokers)	50 mg βC or vitamin E (400 mg) every other day ± aspirin	No βC effect on cancer rates nor on CVD; CVD death decreased 24% with vitamin E; no change in cancer, MI, or stroke; high fruit and vegetable intake associated with lower MI
GISSI—Italy (1999) [138]	11,324 MI survivors, ∼85% male (3−5 years)	Vitamin E (300 mg) ± PUFA (1 g) daily	No effect of vitamin E on CVD; PUFA effects protective; cardiovascular deaths: −30%; mortality: −20%
ASAP—Finland (2002) [125]	520 high-cholesterol male and female (postmenopausal) smokers and nonsmokers (6 years)	Vitamin C (250 mg) and 91 mg α-tocopherol, twice daily	Carotid atherosclerosis −25% combined, −30% in men, decreased plaque size in >50%
IVUS—United States (2002) [126]	40 cardiac transplant patients (1 year)	Vitamin C (500 mg) and vitamin E (263 mg) twice daily	Decreased atherosclerotic plaque size
British Heart Protection Study—United Kingdom (2002) [127]	20,536 increased risk of MI; 15,454 males, 5082 females (5 years)	Vitamin C (250 mg), vitamin E (600 mg), and 20 mg βC	No effect on cancer, stroke, or dementia
SU.VI.MAX—France (2003) [82−85]	12,741 men (aged 45−60 years) and women (aged 35−60 years) (7.1 years)	βC (6 mg), vitamin E (30 mg), vitamin C (120 mg) + Zn (20 mg), and selenium (100 μg) daily dosing	Protective in men: prostate cancer: ∼ − 58%; all cancer: −31% and mortality: −37%; no supplement effect in women but lower CVD with high fruits and vegetables
HOPE & HOPE-TOO—Canada, United States, Europe, South America (2005) [139,140]	1138 men and women (over 55 years) with left ventricular dysfunction or diabetes (4 years + follow-up to 7 years)	Vitamin E (400 mg) daily	No significant effect on cancer incidence or mortality; no significant effect on CVD events but heart failure +13%

(*Continued*)

TABLE 16.3 (Continued)

Study and Publication Date	Study Details, Size, and Duration	Intervention Details	Study Results
E3N Prospective Cohort Study—France (2006) [74]	59,910 women—selected 700 with smoking-related cancers (7.4 years)	βC intake divided into quartiles from diet reports	Nonsmokers 20% to 60 + % reduction in lung cancer; smokers 1.5−2X increase
SELECT—United States (2008) [66]	35,533 men (5.46 years); PSA <4 ng/mL and normal digital exam (aged 50 or 55 years) (5.5 years)	Se 200 μg/day as selenomethionine, racemic vitamin E 400 IU/day	No significant effect on prostate cancer or diabetes for combination, nonsignificant increase in prostate with vitamin E
PHS II—United States (2008) [65]	14,641 male physicians (50 years or more) + 8 year follow-up	Vitamin C (500 mg daily) vitamin E 400 IU every other/day	No significant effect on lung, colorectal, prostate, or total cancer; vitamin E-associated hemorrhagic stroke
WASC—United States (2009) [64]	7627 cancer-free women (40 years or more) (9.4 years)	Vitamin C (500 mg daily) and vitamin E 600 IU + βC (50 mg) every other/day	No significant effect on total cancer incidence or cancer mortality

individuals, perhaps at untoward high doses or in the presence of specific conditions or cancer treatments, may modulate normal protective benefits, whereas others may bring about deleterious effects, such as interfering with the efficacy of cancer drug treatment or increasing an individual's risk for cancer or heart disease [50,51].

1 Antioxidant Supplementation and Cancer Prevention

On initial examination of Table 16.3, large-scale trials do not consistently demonstrate a definite benefit of antioxidant supplements in healthy individuals. However, closer examination suggests that in individuals with initially low background antioxidant status, supplementation to achieve a normal range may bring about some health benefit [52]. In some cases, however, high-dose supplementation may lead to potentially harmful elevated blood levels, especially in oxidatively stressed individuals such as smokers who consume large amounts of β-carotene. This subject was further examined in a recent review by Yang et al. [53] focused on vitamin E, selenium, and β-carotene.

a β-Carotene

A major issue in assessing antioxidant efficacy is whether any antioxidant interventions, especially at elevated doses above the normal nutritional level, result in a dose-dependent benefit to individuals at risk from disease. For example, two large-scale, randomized intervention trials evaluated the effect of β-carotene supplementation over 12 years on the primary prevention of cancer and CVD in male physicians (Physicians Health Study [PHS]) [54] and over 2 years in female health professionals (Women's Health Study [WHS]) [55]. The dose administered was 50 mg β-carotene every other day. Upon initial evaluation, no evidence of either stimulation or inhibition of either disease was noted when placebo and treated groups were compared. However, subgroup analyses of the PHS data later found that men with the lowest quartile for plasma β-carotene at initial baseline had a lower risk for total cancer, particularly prostate cancer, when β-carotene supplement users were compared with placebo [56]. The average blood levels achieved during the PHS were 120 μg/dL [57], whereas normal serum β-carotene is in the range of 5−50 μg/dL [1]. An effect on smokers was not observable in these two studies, possibly because only 11% of the physicians and 13% of the women were smokers; therefore, the study was not adequately powered to examine this aspect. There is no reason to suspect that these individuals were nutritionally compromised, so the results may not be universally applicable to the general population. Additional β-carotene findings have been derived from nested cohort subsets of randomized clinical trials of β-carotene. These studies found no evidence that β-carotene supplementation prevented recurrence of basal or squamous carcinomas of the skin (50 mg/day) in the United States [58] or of colorectal adenomas (20 mg/day) in Australia [59]. However, rather recent meta-analyses of circulating carotenoids [60], carotenoid intake [61], and fruit and vegetable intake [62] and an European Prospective Investigation into Cancer and Nutrition (EPIC)-based plasma antioxidant (carotenoids, tocopherols, retinol, and Vitamin C) study on breast cancer risk [63] all indicate increased antioxidants (carotenoids and vitamin C) are associated with lower ER-breast cancer incidences. These recent studies give support to further

examination of metabolic effects of smoking and the metabolomics of food-based carotenoids and vitamin C, both areas of research drastically slowed by the two results of studies of high dose in smokers.

Similarly, several randomized trials in high-risk populations have reported reduced disease risk using antioxidants, at least in baseline deficient populations when subsets of the populations are examined. The National Cancer Institute (NCI) trials, conducted in Linxian, China, with a population at high risk for esophageal cancer, noted a significant benefit for those receiving a β-carotene/vitamin E/selenium combination: a 13% decrease in the cancer mortality rate, a 21% decrease in stomach cancer mortality, a 4% decrease in esophageal cancer mortality, a 10% decrease in deaths from strokes, and a 9% decrease in deaths from all causes [52]. However, the generalizability of these findings from Linxian may not be universally appropriate because individuals in this study appear to have limited intakes of several micronutrients. Thus, these prevention agent trial findings may not be applicable to well-nourished populations. Indeed, several studies did not show stimulatory or inhibitory effects on total cancers or the target organs in U.S. populations [64–66] (Table 16.3).

2 Effects in Smokers

When smokers were selected as the target population, very different views surfaced about the health consequences of β-carotene supplements. Two highly publicized trials provided evidence for potentially adverse effects of antioxidants, particularly in smokers and in individuals exposed to certain environmental hazards, such as asbestos. These two independent, randomized, clinical trials—the 5- to 8-year ATBC (Alpha-Tocopherol Beta-Carotene) [67] and the 4-year CARET (Beta-Carotene and Retinol Efficacy Trial) [68]—reported adverse effects of 20- and 30-mg β-carotene daily supplementation on lung cancer risk in these high-risk populations. The ATBC trial studied effects primarily in smokers, whereas the PHS included more than half who were nonsmokers [54]. Similar to the PHS results for β-carotene, the ATBC study data demonstrated a reduction (32%) in prostate cancer incidence in the α-tocopherol (vitamin E) supplemented group [69] and 22% fewer cases of colorectal cancer [70] upon reanalysis. Although lung cancer incidence was elevated in both ATBC and CARET smokers with β-carotene, after a 12-year follow-up of CARET participants, a significant decrease in lung cancer incidence in the placebo arm was observed that was linked to fruit and vegetable intake when the lowest versus highest quintiles were compared [71]. An earlier case–control study of lung cancer in nonsmokers conducted in New York concluded that dietary β-carotene, raw fruits and vegetables, and vitamin E supplements reduced the risk of lung cancer in nonsmoking men and women [72]. A subsequent evaluation of the Nurses' Health Study from Harvard implicated carrots but not β-carotene, further adding to the confusion [73]. Interestingly, in the French Etude Epidemiologique de Femmes de la Mutuelle Generale de l'Education Nationale (E3N) prospective investigation, the nonsmoking women showed a dose-dependent lowering of lung cancer risk when intakes were considered. The largest reduction was observed in women taking β-carotene supplements, but smokers showed the commonly observed increased risk [74]. Research has implicated β-carotene cleavage products as likely factors in the increased cancer activity of high β-carotene doses, exacerbated by smoking-induced alterations in β-carotene metabolism [57,75,76].

3 Follow-Up Examinations of Existing Studies

Follow-up examinations of a subset of the ATBC cohort have reported a marked association between elevated serum levels of α-tocopherol and reduced prostate cancer risk in a 6-year prospective study with 100 prostate cancer patients and 200 controls [77]; however, a separate postintervention study reported that the excess risk for lung cancer and the beneficial effect for prostate cancer were no longer significantly different from controls [78]. In addition, with the exception of a slight carotene protective effect on early stage laryngeal cancer, other upper aerodigestive cancers were not affected [79], although a dose–responsive decrease in mortality with increasing vitamin E serum levels was noted [80]. Interestingly, evaluation of dietary records in the ATBC study indicated that consumption of fruits and vegetables was associated with a lower lung cancer risk, as were the levels of dietary lycopene and other carotenoids, as well as actual serum levels of β-carotene and retinol, when the highest and lowest baseline quintiles were compared [81]. A 6-year follow-up of the CARET cohort reported that the increased risk was no longer significant for either lung cancer or CVD, but subgroup analysis suggests excess risk in heretofore unreported susceptible groups (females and former smokers), a difference from the ATBC study that may be due to the higher β-carotene dose in CARET [47] or perhaps the presence of vitamin E (α-tocopherol). The return to normal risk levels in the smokers suggests that subtle changes were introduced such that this group may have actually achieved protection from prior treatment. Thus, it is conceivable that the increased risk observed in the ATBC study may have reflected an increase in those with a preexisting precancerous lesion or tumor but protection in those without. This hypothesis would also explain the lack of increased cancer in the Women's Antioxidant Cardiovascular Study, in which cancer-free women were given vitamins C and E plus

50 mg β-carotene [64]. Thus, the question of benefits or merits of β-carotene in smokers remains controversial because it may depend on the presence or absence of precancerous lesions.

Further follow-up and reexamination of subgroup analysis may also help explain these unexpected results. For example, the French study, SUppléments en VItamines et Minéraux AntioXydants (Antioxidant Vitamin and Mineral Supplements [SU.VI.MAX]), examined the effects of supplementation with vitamins C and E (120 and 30 mg, respectively), 6 mg β-carotene, 100 μg selenium, and 20 mg zinc on the health of approximately 13,000 men (45−60 years) and women (35−60 years) [82−85]. In men only, antioxidant supplementation reduced the risk of developing all types of cancers by 31%. This effect was most pronounced in men with low baseline levels of β-carotene who had the greatest increased risk of developing cancer. An evaluation of the study data showed that there was a nonsignificant reduction in prostate cancer rate associated with the supplementation in all men, but there was a significant difference between men with normal baseline prostate-specific antigen (PSA) and those with elevated PSA. Among men with normal PSA, there was a statistically significant reduction in the prostate cancer rate among those receiving the supplements. Surprisingly, the supplementation was associated with an increased incidence of prostate cancer, with border-line statistical significance in men with elevated PSA at baseline [85]. The average β-carotene blood levels both at the beginning of the study (∼39 μg/dL in women and ∼25 μg/dL in men) and at the end (∼90 μg/dL for women and ∼50 μg/dL for men) were below those seen in the PHS, whereas no such change was observed for serum levels of vitamins E or C, selenium, or zinc.

a Selenium

Dietary selenium is another potentially important antioxidant and potential chemopreventive agent because of its importance in the functionality of glutathione peroxidase. Strong evidence linking selenium supplementation and decreased cancer risk comes from the secondary analysis of the Nutritional Prevention of Cancer (NPC) trial, a study designed for patients with a history of basal or squamous cell carcinomas [86]. Although no benefit was observed for skin cancer, the primary end point of the study, secondary end point analyses showed significant reductions in relative risk for total cancer mortality (50%), total cancer incidence (37%), as well as incidences of lung (46%), colorectal (58%), and prostate (63%) cancer for patients who received selenium supplements. Further reanalysis of the NPC data confirmed a 49% reduction in prostate cancer incidence in men receiving selenium supplements compared to controls, although follow-up suggests the benefits may be decreasing with time [87]. Another reanalysis that included three additional years of follow-up data found a nonsignificant decrease in lung cancer risk for all patients who received selenium supplements; however, the analysis suggested a significant risk reduction following supplementation among those with the lowest baseline plasma selenium [88]. A separate study by Li et al. [89] also indicated that a reduction in prostate cancer incidence occurred following selenium supplementation. The authors of these various studies differ in their conclusions concerning whether selenium prevents initiation of carcinogenesis or inhibits tumor progression; nevertheless, the benefit of selenium for prostate cancer prevention is fairly consistent. A wealth of preclinical studies supports the anticancer properties of selenium. Much of the evidence concerning selenium's anticancer/antitumor properties suggests mechanisms of action other than those associated with its antioxidant properties [90]. A clinical trial of selenium as selenomethionine in combination with vitamin E, however, failed to show a significant effect on prostate cancer or diabetes, but selenium alone gave a nonsignificant increase in diabetes and a similar effect on the prostate in a very large cohort of relatively healthy U.S. men [66].

4 Antioxidant Combinations

More broadly, a meta-analysis of antioxidant supplementation in patients at elevated risk of gastrointestinal cancers revealed no protective effect for vitamins A, C, and E on esophageal, gastric, colorectal, pancreatic, and liver cancers. However, selenium supplementation was noted as possibly a cancer deterrent. The authors have noted that the findings may not be easily extrapolated to the effects of fruits and vegetables, which are rich sources of only some of these antioxidants [46]. Likewise, the applicability of these findings to healthy individuals is unclear. The follow-up examination of fruit and vegetable use and breast cancer risk within the EPIC study indicated that fruits and vegetables had no effect on cancer when the highest versus the lowest quintiles were compared [91]. These findings add to the confusion in light of other studies that suggest fruits and vegetables have benefits, particularly when associated with their antioxidant content [71,72,74]. The WHS, however, saw a reduction in myocardial infarction (MI) [92]. These inconsistencies may reflect the variation in the intake of individual fruits and vegetables, their interactions with environmental insults, or the genetic background of the consumer.

Completion of the human genome sequence and the advent of DNA microarrays using cDNAs enhanced the detection and identification of hundreds of differentially expressed genes in response to antioxidants including

flavonoids, selenium, zinc, and several vitamins [93]. The phenolic antioxidant resveratrol found in berries and grapes has been reported to inhibit the growth formation of prostate tumor cells by acting on the regulatory genes such as p53 while activating a cascade of genes involved in cell cycle and apoptosis, including p300, Apaf-1, cdk inhibitor p21, p57 (KIP2), p53-induced Pig 7, Pig 8, Pig 10, cyclin D, and DNA fragmentation factor 45, although some findings were not confirmed by reverse transcriptase polymerase chain reaction [94]. Likewise, the expression of a host of genes is influenced by selenium supplementation [95].

B Antioxidants and CVD

While the safety of antioxidants and potential for efficacy in vivo and in vitro studies has been well established [50], clinically based studies have not been as promising for a number of antioxidant compounds including vitamin C, vitamin E, resveratrol, epigallocatechingallate, a tea polyphenol (EGCG), lycopene and other carotenoids, and coenzyme Q10. Meta-analysis of clinically based studies of vitamin E supplementation showed increased mortality above a 400-mg dose [96], compared to a protective effect of fruit and vegetable consumption in EPIC [91] and a meta-analysis of fruit and vegetable cohort studies [97], thus providing mixed evidence. A dose-dependent inverse relationship between vitamin E consumption and coronary heart disease mortality was detected in the prospective Iowa Women's Health Study [98]. The strongest inverse association was observed among the approximately 22,000 women who did not consume vitamin supplements compared to those who did [98]. Vitamin E meta-analyses showed no benefit of vitamin E supplementation, but high doses significantly increased overall mortality [99–101].

The meta-analysis of high-dose vitamin E supplementation by Miller et al. [96], however, suggested a positive association of all-cause mortality when supplementation was above 400 IU/day. Despite a number of issues with the choice of studies to be included and excluded in the meta-analysis, as well as the failure to consider a dose–response relationship in the physiological range, Wright's earlier analysis of continuous serum α-tocopherol values in the ATBC study indicated a dose-dependent reduction in mortality from chronic disease with increasing concentrations up to approximately 13 or 14 mg/L (30–33 mmol/L), after which no further benefit was noted. Those with the higher baseline blood levels showed decreased mortality [80]. The use of superphysiological exposures with questionable health benefits, especially for those at the highest status, raises serious concerns about the wisdom of megadoses of vitamin E supplements. A meta-analysis of more than 60 clinical trials that focused on antioxidant use (including vitamin E) reached similar conclusions about slightly increased mortality but again may have introduced some biases in the evaluation [102]. Another study, the Canadian Heart Outcomes Prevention Evaluation (HOPE)—a randomized controlled prospective investigation of vitamin E supplementation (400 IU/day) in approximately 4700 patients with diabetes and other cardiovascular risk factors—linked supplementation with an increased risk of heart failure but no protection from cancer in the 2.6-year follow-up portion of the study; no differences in cardiovascular effects were noted during the first 4.6 years [103]. More specifically, vitamin E supplementation of 1200 IU/day was associated with a 50% increase in chronic heart failure (CHF) in patients with left ventricular (LV) dysfunction, and despite improvements in plasma levels of markers of inflammation such as high-sensitivity C-Reactive protein and levels of oxidative stress such as oxidized LDL, there was no change in intima media thickness of carotid arteries and no significant change in CV events [104,105]. There is a strong inverse association between lycopene intake and incidence of MI, angina pectoris, and coronary insufficiency. Additionally, low lycopene levels were reported by many researchers in hypertension, MI, stroke, and atherosclerosis [106]. However, when supplementing with lycopene, there are less convincing results [107].

Research evidence from large-scale trials also indicated no clear benefit of antioxidant supplements in healthy individuals when considered in toto. Upon closer examination, supplementing individuals with low background levels to a normal range may be beneficial, but high-dose supplementation leading to markedly elevated blood levels may be harmful, especially in oxidatively stressed individuals, such as smokers in the case of β-carotene or in unhealthy or older patients in the case of vitamin E.

1 Targets of Antioxidant-Related Disease Protection

Vitamin E, vitamin C, and β-carotene intake, whether by supplementation or as components of foods, has been extensively studied for their potential to reduce CVD or conditions related to the sequelae of CVD and other vascular diseases. A number of older studies suggest that vitamin E, alone or in combination with other antioxidants, may be protective against atherosclerosis in at-risk populations. In the WHS, there was a significant (24%) reduction in cardiovascular deaths [108] in the vitamin E group. However, other studies question the overall benefits [67,109–111]. In addition to a potential role in minimizing oxidative DNA damage and lipid peroxidation, vitamin E may modify several other functions, some of which specifically protect against processes known to contribute to atherosclerosis, including inhibiting protein

kinase C activity and smooth muscle cell proliferation, inhibiting cell adhesion and platelet aggregation, counteracting inflammation, and enhancing bioavailability of nitric oxide to improve endothelium-dependent vasodilator function [112–114]. Vitamin E may also improve insulin-mediated glucose uptake, thus possibly decreasing the risk for type 2 diabetes, a condition that also contributes to atherosclerosis [115].

Endothelial dysfunction is the major target for vitamin C-mediated effects. In patients with coronary artery disease or hyperglycemia-induced impairment of vasodilation, NO-mediated vasodilation is restored with either oral (6 g over 2 days) or intraarterial infusion (24 mg/minute for 10 minutes) of vitamin C [116]. Vitamin C enhances nitric oxide bioavailability which is essential to preserve endothelial homeostasis and prevents nitrate tolerance [117,118]. Vitamin C supplementation also counteracts endothelial dysfunction which is a contributor to CVD [119]. The EPIC Norfolk study found that plasma vitamin C concentration was inversely related to incidence of CVD-related mortality, as well as all-cause mortality, in both women and men. By increasing plasma vitamin C by 20 mcg through increased intake of vegetables and fruit, CVD mortality was reduced by ~20% [120]. Diets high in vitamin C are associated with decreased stroke risk in those over 65 years old but no significant association occurs with coronary artery disease [121].

The PHS II studied the effects of vitamins E and C in prevention of CVD in men during a mean follow-up of 8 years. The trial did not find any benefit from antioxidant supplementation on major CVD outcomes. Additionally, vitamin E was associated with an increased risk of stroke [122]. Wannamethee et al. reported that higher vitamin C plasma levels are inversely associated with cardiovascular risk factors including blood lipid and blood pressure, but supplementation of Vitamin C did not exert any influence and has not been shown to have benefit in reducing major cardiovascular events [123], additionally, the dietary intake of vitamin E was significantly correlated with increased risk of heart failure [124].

2 Clinical Trial Interventions for CVD Amelioration

Antioxidants have been tested for the ability to ameliorate development of CVD in some at-risk populations. The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study, a 6-year randomized trial, supplemented 520 hypercholesterolemic men and postmenopausal women (45–69 years old) with vitamin C and vitamin E [125]. A significant decrease (26%) in the progression of carotid atherosclerosis was observed in men, although no significant effect was seen in women. In the Harvard Intravascular Ultrasonography (IVUS) study, a combination of vitamin C and vitamin E was given to 40 (35 males) cardiac transplant patients. Cardiac transplantation leads to oxidative stress, which may contribute to atherosclerosis; supplementation of patients with these vitamins slowed the progress of coronary atherosclerosis [126].

Two large studies have reported preliminary results. The British Heart Protection Study, a multicenter, randomized, double-blind, placebo-controlled trial enrolled 20,536 patients aged 55–75 years who were at an increased 5-year risk of MI [127]. Participants received antioxidant vitamins (combination of 600 IU vitamin E, 250 mg vitamin C, and 20 mg β-carotene) and simvastatin or each separately. No adverse or beneficial effect on vascular or nonvascular morbidity or mortality could be attributed to supplementation with vitamins. The American PHS II enrolled 15,000 physicians aged 55 years or older in a randomized, double-blind, placebo-controlled trial to test β-carotene, vitamin E (400 IU synthetic on alternate days), vitamin C (500 mg/day), and multivitamin (Recommended Dietary Allowance [RDA] of most vitamins and minerals) [128]. This study was completed in September 2007 and investigated the effects of supplementation on prevention of total and prostate cancer, CVD, as well as age-related eye diseases (cataracts and macular degeneration). No effects on cancer were observed. Preliminary analysis reported no significant effect of any of the vitamins on cardiovascular outcomes, but a greater number of hemorrhagic strokes were observed in the vitamin E versus placebo group; the effects of multivitamin use are still being evaluated [65]. The WHS tested the ability of aspirin, vitamin E, and β-carotene to prevent cancer and CVD [55,108]. Analysis of β-carotene supplementation showed no effect of β-carotene on risk of CVD. In the vitamin E group, there was a significant (24%) reduction in cardiovascular deaths [108]. Despite the lack of evidence for an effect of the combined discrete nutrients on CVD, high fruit and vegetable intake was associated with lower risk for MI in the WHS study [92].

As seen in the WHS, dietary habits have been linked to decreased CVD risk, despite a lack of effectiveness for individual supplements. Similarly, Mennen et al. [129] found that a diet rich in flavonoids reduced CVD risk in a subset of women participating in the SU.VI.MAX study [82–85]. Dietary intakes were estimated using six 24-hour dietary records collected during the course of 1 year. In women, flavonoid-rich food consumption was associated with decreased systolic blood pressure and a decreased risk for CVD; this relationship was not observed in men. The lack of an effect in men could be attributed to the men's higher risk for CVD. However, inconsistencies in dietary reporting and measuring of

flavonoid consumption could also contribute to this discrepancy. Still, this study found that after 7.5 years of follow-up, no protective effect against ischemic heart disease attributable to antioxidant supplementation could be discerned in either men or women [129].

Multiple studies have reported a strong inverse relationship between lycopene and carotenoid intake and incidence of MI, angina pectoris, coronary insufficiency incidence, and stroke risk [130–132]. Additionally, low lycopene levels were reported by many researchers in hypertension, MI, stroke, and atherosclerosis [133]. Higher adipose lycopene concentrations correlated with a reduction in risk of MI and the effect was retained after correction for confounding factors. The odds ratio was found to be 0.52 suggesting the risk of mortality was substantially reduced in those with high-adipose lycopene [134]. Although lycopene from dietary sources has extremely low bioavailability [135], natural dietary sources are recommended as supplemental lycopene has not demonstrated the same cardiovascular protection as that of lycopene obtained from the diet [107,136].

Four large, randomized clinical trials specifically tested the ability of vitamin E supplementation to slow the progression of CVD in individuals at increased risk for CVD death (reviewed by Salonen [137]). The ATBC trial, originally designed to test cancer prevention abilities of vitamins C and E and β-carotene, tested supplementation with 50 mg α-tocopherol acetate daily for 5–8 years [67]. In this study of male smokers, α-tocopherol supplementation was associated with a modest trend toward decreased incidence of angina pectoris but no decrease in CVD mortality. β-Carotene supplementation had no preventive effect and was associated with a slight increase in the occurrence of angina [109]. An apparent excess of hemorrhagic stroke in the treatment group complicated this study. The Cambridge Heart Antioxidant Study (CHAOS) tested RRR-α-tocopherol from "natural sources" in 2002 participants with clinical evidence of CVD [110]. Trial analysis identified a 77% decrease in nonfatal MI but also a nonsignificant increase in early deaths from CVD and total mortality, although no increase in risk of hemorrhagic stroke was observed. Interpretation of this study was complicated by unbalanced randomization, incomplete follow-up, and a midstudy change in vitamin E dose (800 IU/day to 400 IU/day) [111]. The Italian Gruppo Italiano per lo Studio della Supravvivenza nell Infarto Miocardio (GISSI) study supplemented 11,324 participants with previous MI with 300 mg all-racemic α-tocopherol daily, with or without 1 g polyunsaturated fatty acids (PUFAs) [138]. No effect was observed in the group receiving both supplements, but a significant 20% decrease in cardiovascular death was observed in the group receiving only PUFAs [138]. In the HOPE study, 2545 women and 6996 men at high risk for CVD received either 400 IU/day "natural source" vitamin E or Ramipril; this study found no effect of supplementation on any parameters related to CVD [139,140]. As noted previously, however, the follow-up study suggests an increase in CVD after an additional 2.6 years [103]. The Women's Health Initiative examined multivitamin use in more than 160,000 women during an 8-year clinical trial follow-up period with nearly 8 years of observational follow-up. More than 40% used multivitamins, and this was not linked to increases in several common cancers, CVD, MI, stroke, and overall mortality [141].

ROS can influence the inflammatory process. As inflammation is thought to be a significant cause of damage to blood vessels, contributing to CVD, it has been a logical target for treatment with antioxidants. Vascular smooth muscle cell accumulation and hypertrophy, and nitric oxide regulation of endothelial vasorelaxation and vasodilation, are processes that may also be therapeutically modulated by antioxidants. Although individual supplements have not proven to be strongly effective, diets high in fruit and vegetables, and their native antioxidant and phytochemical combinations, have been shown to be fairly protective [97]. Plant-based diets such as the Mediterranean diet are promising in their ability to prevent cerebrovascular disease and CVD [142]. Of course, one must consider that diets high in fruits and vegetables may indirectly reduce the risk of CVD by promoting healthy body weight, decreasing the risk of developing conditions contributing to CVD such as hyperlipidemia and type 2 diabetes.

Studies with defined populations have generally failed to demonstrate marked prevention potential for cancer or heart disease, but animal studies suggest that benefits should occur. There are a number of possible explanations for this inconsistency. Foremost among these is that significant nutrient–nutrient interactions are likely occurring along with the importance of environmental insults as determinants of the overall response. Some of the case–control studies may actually be selecting participants with a general interest in the pursuit of a healthy lifestyle. It is known that volunteers for observational antioxidant studies tend to have better diets, exercise more, use less alcohol, and come from higher socioeconomic backgrounds, all of which may contribute to a decreased baseline risk for CVD and other disease conditions [143].

C Antioxidants and NDDs

Most of the antioxidant classes shown in Table 16.1 have been studied in relation to NDDs. In particular, multiple reports have focused on carotenoids, curcuminoids, flavonoids, selenium, and vitamins C and E. In various studies these compound classes have been either studied alone, in

combination, or from botanical extracts. Thus, it is often difficult to compare the studies directly to one another. This difficulty is also compounded by differences in subject population, study design, and duration.

A post hoc analysis of the SU.VI.MAX trial in France (see Table 16.3) looked at the effects of supplementation with vitamin C, β-carotene, vitamin E, selenium, and zinc for 6 years on cognitive performance of almost 4500 French subjects [144]. The results showed that subjects receiving the supplements had significantly better scores on some measures of verbal memory (as one of a number of measure of cognitive function). A recent RCT providing cocoa flavonols to a smaller number of subjects for only 8 weeks also suggested that regular consumption of the antioxidant reduced some but not all measures of age-related cognitive decline [145].

More recent work has suggested that lutein, a carotenoid with antioxidant properties, may play a role in maintaining cognitive function in aging. This work grew out of numerous studies demonstrating the selective accumulation of lutein in the eye (where it is a component of macular pigment) and its possible role in protecting against age-related macular degeneration [146]. This led to the finding that lutein is the predominant carotenoid in brain [147]. There is recent work demonstrating that macular pigment density is related to cognitive function and that direct supplementation of the diet with lutein improves some measures of cognitive function in older women [148,149].

Results of studies investigating the effects of single or multiple antioxidant interventions on cognitive function have been mixed, at best. Most studies use multiple measures and the effects, if seen at all, are reflected only in a few. Nonetheless, the potential that a simple dietary intervention or antioxidant supplement can slow the progression of MCI is important and merits further investigation.

More recently attention has turned to assessing the effects of "dietary patterns" as opposed to single or multiple dietary antioxidants. In this regard, other post hoc analyses of the SU.VI.MAX trial have shown provocative results [150,151]. In the first study, adherence to a "Mediterranean Diet" was assessed in 3083 subjects who were also assessed for multiple measures of cognitive function. The results showed no beneficial effects of adherence to the dietary pattern. In contrast, the subsequent study that focused on a "carotenoid-rich dietary pattern" found a significantly higher composite cognitive score for those adhering to that pattern. A recent study of Swedish subjects correlated adherence either to a "prudent diet" or a "western diet" with cognitive performance over time as assessed by the mini-mental state examination (MMSE) [152]. The highest adherence to the prudent diet was related to less MMSE decline, while adherence to the western diet was associated with more decline. Moreover, the decline associated with the western diet was ameliorated by a higher adherence to the prudent diet. Thus, the authors suggested that a prudent diet may alleviate the adverse effects of a western diet on cognitive function.

Given the variability in results in studies of dietary antioxidants and cognitive function in aging individuals without frank dementia, it is not surprising that there is an even more confusing picture on the role of antioxidants in the treatment or prevention of Alzheimer's disease and other dementias. Studies addressing these issues often, and necessarily, involve the use of brain imaging techniques and/or biomarkers of amyloid pathology instead of, or in addition to, measures of cognitive function. For example, the Alzheimer's Disease Cooperative Study, Antioxidant Biomarker Study, involved 78 subjects with mild to moderate Alzheimer's disease [153]. Treatment was for 16 weeks with (1) vitamins E and C and α-lipoic acid, (2) coenzyme Q, or (3) placebo. The antioxidant treatment had no effect on cerebrospinal fluid biomarkers of amyloid or tau pathology but vitamin E, vitamin C, and lipoic acid treatment accelerated the decline in MMSE scores. In spite of such results, there is still much hope in the use of antioxidants in the prevention, treatment, and management of Alzheimer's disease. There are several recent reviews supporting this optimism [43,44,154]. Some authorities suggest that it is useful to view the role of dietary components in Alzheimer's disease in terms of maintaining adequate antioxidant nutritional status and fulfilling possibly increased requirements [154,155].

D Polymorphism: An Additional Risk Factor in Cancer

Evidence is increasing that genetic polymorphisms can influence the response to an arsenal of agents used in the battle against cancer, including both drugs and dietary components. However, these investigations are often conflicting because the development of cancer is not a simple process but, rather, involves multiple cellular events, many of which are likely influenced by genetic polymorphisms at the site of the target or by how the agent is modified through absorption, metabolism, or excretion.

Although ROS are integral to many cellular and biomolecular processes associated with acute coronary syndromes, metabolism, and early stages of cancer, the relationship to specific genetic polymorphisms has not been overly compelling and remains controversial. It may be that current eating behaviors prevent the easy identification of diet−gene−health interactions in this disease condition. However, mechanistically, the linkage with free radical-related gene polymorphisms is logical and deserves additional attention as a potential, subtle

long-term regulator of both cancer and heart disease risk. Little attention has been given to genes associated with oxidative stress and heart disease; the primary focus has been on cholesterol homeostasis and control [156]. However, two papers have focused on ROS and antioxidant vitamins. The overall view is that ROS are strongly linked with atherosclerosis, hypertension, and congestive heart failure, but a demonstrated efficacy of antioxidants has proved elusive [157,158].

A number of scientists have been exploring the effects of genetic polymorphisms on oxidative stress or the ability of antioxidants to influence cancer. Several examples concerning the possible utility of using specific polymorphisms as predictors of cancer risk have surfaced. For example, the manganese superoxide dismutase (MnSOD) protein is involved in decreasing the levels of superoxide anion generated during normal biochemical processes. A polymorphism that changes an alanine residue to valine in position 9 or valine to alanine in position 16 in the protein has been suspected to be a risk factor in prostate and breast carcinogenesis and has been found with higher frequency in individuals with prostate cancer [159–161] and, in some studies, with increased severity of the disease and earlier onset [161,162]. Likewise, breast cancer risk was observed to be slightly increased (odds ratio 1.3) in women with Ala/Ala genotype compared to those with Val/Val genotype [163]. Interestingly, patients carrying the Val allele may have a higher prevalence of cardiomyopathy [164]. However, these early findings need to be further investigated because a number of clinical and meta-analysis studies [160–162,165–172] examining the link of these mutations to both cancers are inconsistent, with some having failed to verify an association and others supporting it (Table 16.4). Some studies have shown the association to be primarily in those with low antioxidant consumption [160,170,173].

The catalase (CAT) gene polymorphism at 262C-T may also have a role in breast cancer development. This antioxidant enzyme neutralizes hydrogen peroxide and is known to be induced by oxidative challenges. A 262C-T polymorphism in the promoter region of CAT is associated with risk of several conditions related to oxidative stress. Interestingly, the CC genotype relationship with breast cancer reduction was only observed among Caucasians and not in African Americans [174]. CAT polymorphism at codon 262 does not appear to be related to diabetes and the risk of heart disease [175]. The reason for the disconnect among genetic polymorphisms (CAT and MnSOD), oxidative damage, and risk of cancer and heart disease warrants additional attention but may be due to an enhanced response to antioxidants in the CC genotype [173].

Still another gene involved in antioxidant activity and cancer risk is glutathione peroxidase 1 (GPX-1) [139]. Although codon 198 can lead to leucine or proline, it was determined that the leucine-containing allele was more frequently associated with breast cancer than was the proline-containing allele [176]; however, there are inconsistencies in the literature [177,178]. Combinations of gene polymorphism may offer additional insights into risk. Cox et al. [179] observed an increased breast cancer relative risk (OR = 1.87) in individuals with the MnSOD Ala16Ala genotype and the Leu198Leu genotype of GPX-1, whereas neither surfaced as risk modifiers when considered independently.

Environmental factors may also dictate the importance of genetic polymorphisms in determining cancer risk. For example, the T allele in the GPX-1 gene at position 198 is considered to be protective in smokers. In a study of smokers with 432 lung cancer cases and 366 controls, those possessing the variant T allele were significantly less likely to develop lung cancer than individuals without it [180]. Likewise, patients with alcohol-induced cirrhosis who had the genotype consistent with low MnSOD activity and also with high GPX-1 activity had much lower levels of potentially toxic levels of iron. In individuals

TABLE 16.4 Meta-Analyses of Prostate and Breast Cancer Risk with SOD Mutations

Mutation	Organ	No. of Studies	Cases	Controls	Results	Reference
Ala-9Val	Prostate	5	889	1841	Nonsignificant increase	[171]
Ala-9Val	Prostate	9	1660	2594	Positive	[160]
Ala-9Val	Breast	13	4278	5057	Nonsignificant increase	[160]
Ala-16Val	Prostate	4	2379	4066	Nonsignificant increase	[171]
Ala-16Val	Prostate	32	26,022	32,426	Negative	[172]
Ala-16Val	Prostate	12	3574	5388	Positive	[169]
Ala-16Val	Breast	17	9710	11,041	Negative	[165]

with both polymorphisms, none developed hepatocellular carcinoma, as opposed to other polymorphism combinations, for which 16−32% of the individuals developed liver cancer [181]. In addition, consumption of a number of food items may influence the relationship between individual polymorphisms and health. Both aldehyde dehydrogenase-2 (ALDH2) and X-ray repair cross-complementing 1 (XRCC1) genes have been identified as having a role in the response to dietary selenium. The glutamic acid 487 lysine polymorphism in the ALDH2 gene and the arginine 399 glutamine polymorphism in the XRCC1 gene are both associated with an increased risk of esophageal cancer in individuals consuming a low-selenium diet. In addition, this risk becomes even more pronounced in individuals who smoke or consume alcohol [182]. This may reflect the individual differences in the pharmacokinetics related to handling supplemental selenium. Likewise, the protective effect of the catalase CC genotype was even more pronounced among women who used dietary supplements, as well as those with high fruit and vegetable intakes [183]. In another study, an inverse association between fruit and vegetable consumption and breast cancer risk was observed among women with the wild-type genotype for codon 84 of their O6-methylguanine-DNA methyltransferase (MGMT) gene [184]. In this group, the observed OR was 0.8, whereas for individuals with other polymorphisms, the effects of a varied intake were not statistically significant. The association between fruit and vegetable consumption and reduced breast cancer risk was also seen in individuals with a variant allele for codon 143 in their MGMT gene. In another study, the intake of α- and β-carotene as modifiers of skin cancer was found to relate to MnSOD V16A polymorphism, such that an inverse association of intake was limited to the Val carriers, whereas no association was observed among women with the AA genotype [185].

E Antioxidants: Prevention Versus Treatment in Clinical Applications

As has been indicated, antioxidants can have both beneficial and deleterious effects on disease. In the case of CVD generally and atherosclerosis in particular, the effects of antioxidants would be expected to be primarily beneficial because lipid oxidation and inflammation are important factors in the sequelae involving atherosclerotic plaque development. The overall consensus is that interventions with dietary antioxidants seem to have a minor effect on prevention of atherosclerosis [186]. However, reviews on the relationships of statin drugs with free radical generation and inflammation provide evidence that statin drugs have a strong protective effect over and above their more well-known cholesterol-lowering activity [187]. Some of these protective effects, however, may be due to increased expression of antioxidant enzymes such as catalases and a suppression of pro-oxidant enzyme systems that reduce the production of the free radicals superoxide and peroxynitrite [188,189].

Antioxidant and ROS effects on the cancer process are in part dependent on the status and behaviors of the consumer as well as the stage of development and type of cancer involved. The ability to predict and distinguish between the positive and negative effects of antioxidants is especially crucial in those receiving cancer treatments.

Many cancer patients take vitamin supplements for protection and health promotion. A survey of patients at a comprehensive cancer center found that 60% of patients used vitamins and the majority combined them with conventional therapy [190]. Similarly, among a Massachusetts cohort of women with early stage breast cancer, 60% used megavitamin therapy along with surgery, chemotherapy, and/or radiation therapy [191]. Given the large numbers of cancer patients taking antioxidant vitamins, with or without the knowledge of their oncologists, development of a greater understanding of the actions of antioxidants within the cancer disease and treatment milieu is crucial. Consumer awareness of the issues between antioxidants and cancer appears to be increasing but is far behind other health-related dietary practices [192].

Radiotherapy, as well as many chemotherapeutic agents, eliminates cancer cells by inducing apoptosis via generation of ROS. Thus, it was feared that antioxidants, especially in megadoses, may decrease ROS production and thus decrease the benefits of these treatments. Nevertheless, a number of in vitro and some clinical studies have shown that antioxidant treatment during standard cancer therapy does not always interfere with these therapies. The use of high-dose levels of antioxidants appears to be critical to achieve a beneficial outcome when antioxidants are used with more conventional treatments. In several reported studies, low doses of antioxidants stimulated the growth of human cancer cells in culture, and a single low dose of antioxidant before radiation therapy protected cells against radiation damage, suggesting that low doses of antioxidants might have detrimental effects on cancer treatment [193,194]. High-dose levels have been defined in humans as up to 10 g vitamin C/day, up to 1000 IU vitamin E/day, and up to 60 μg β-carotene/day; in tissue culture, high levels have been considered up to 200 μg vitamin C/mL, up to 20 μg vitamin E/mL, and up to 15 μg β-carotene/mL [195]. Low-dose levels have been defined in humans as approximately the RDA values and in tissue culture as up to 50 μg vitamin C/mL, up to 5 μg vitamin E/mL, and up to 1 μg β-carotene/mL [193].

Although antioxidants may cause some inhibition of ROS production caused by chemo- or radiation therapy, at high levels the antioxidant can directly inhibit tumor growth and can appear to stimulate the drug effects. Prasad et al. [195] described the ability of the α-tocopherol succinate form of vitamin E to enhance the cytotoxic effects of adriamycin in human prostate cancer, glioma, and HeLa cells; enhance cisplatin, tamoxifen, and decarbazine in human melanoma and parotid acinar carcinoma cells; and promote doxorubicin to inhibit murine leukemia cell lines.

The subject of antioxidants during cancer care has been reviewed [196], but data from large-scale trials with antioxidants are not available. Interestingly, some smaller studies provide limited evidence that antioxidant supplementation can increase the efficacy of standard chemotherapies in humans. In 18 nonrandomized patients with small cell lung cancer, supplementation with multiple antioxidants along with chemotherapy or radiation therapy resulted in increased survival times. Retinoic acid and interferon enhanced the effect of radiation on locally advanced cervical adenoma, again improving survival times [197]. Two patients with advanced epithelial ovarian carcinoma (stage IIIC) received antioxidant therapy (1200 IU vitamin E, 300 mg coenzyme Q10, 9000 mg vitamin C, 25 mg mixed carotenoids, and 10,000 IU vitamin A) before conventional chemotherapy and 60 g ascorbic acid twice weekly at the end of therapy. Both women had normal CA-125 levels and remained disease-free more than 3 years after initial diagnosis [198]. In an open trial in patients with small cell lung cancer, patients who received individualized daily supplements of trace elements, fatty acids, and vitamins (including 15,000−40,000 IU vitamin A, 10,000−20,000 IU β-carotene, 300−800 IU vitamin E, 2−5 g vitamin C, and 1600−3400 μg selenium) had a greater 2-year survival rate than historical controls (33% vs 15%) [199,200]. Furthermore, an analysis of 385 breast cancer patients asked to recall antioxidant supplement use showed that users of supplements were less likely to have a breast cancer recurrence or breast cancer-related death than nonusers [201]. In contrast, an analysis of outcomes for 90 women with unilateral nonmetastatic breast cancer who received megadoses of vitamins and minerals (β-carotene, vitamin C, niacin, selenium, coenzyme Q10, and zinc) showed no improvement in survival compared to matched controls (the 5-year survival rate was 72% for cases and 81% for controls) [202]. Although definitive conclusions cannot be drawn from this limited number of studies, improvement in survival of patients receiving antioxidants in addition to chemotherapy has been observed. The use of antioxidant vitamins in conjunction with standard therapy warrants additional attention [203].

III OVERALL CONCLUSION AND DISCUSSION

Excessive oxidative stress can lead to excessive ROS and oxidative free radicals. These in turn can attack proteins, lipids, and genetic material, leading to decreases in immune competence and increased inflammatory response, all linked to several chronic diseases, cancer, CVD, obesity, diabetes, and so on [32]. However, ROS is a potential double-edged sword in disease promotion and prevention. Whereas the generation of ROS once was viewed as detrimental to the overall health of the organism, advances in research have shown that ROS plays crucial roles in normal physiological processes, including response to growth factors, the immune response, and apoptotic elimination of damaged cells. Notwithstanding these beneficial functions, aberrant production or regulation of ROS activity has been demonstrated to contribute to the development of some prevalent diseases and conditions, including cancer and CVD. Antioxidant supplementation has historically been viewed as a promising therapy for prevention and treatment of these diseases, especially given the tantalizing but sometimes conflicting links observed between diets high in fruits and vegetables (and presumably antioxidants) and decreased risks for cancer and CVD. Trials of individual antioxidants, however, have rarely shown strongly beneficial effects. In most healthy individuals, endogenous antioxidant defenses may be sufficient, and extra supplementation may have little effect on disease susceptibility. In some populations, particularly those with underlying illness or compromised nutritional status, dietary or other supplementation may be helpful. A better understanding of the impact of supplementation on disease risk may also be furthered by increased knowledge of individual genetic polymorphisms related to the metabolism and detoxification of ROS and antioxidants, as well as interactions of metabolic intermediates. A more critical issue is the interaction of antioxidants and orthodox cancer therapy.

Advances in tools used to determine oxidative status may allow for the estimating of an individual antioxidant profile. Whether supplements have positive or negative effects may depend on an individual's baseline antioxidant and ROS levels. Antioxidant supplementation in those with low baseline ROS could be detrimental because it may impair normal immune function and prevent ROS-mediated apoptotic elimination of precancerous or cancerous cells. Similarly, better measurements of ROS status may help to predict the potential for antioxidants in individuals exposed to specific environmental toxins, including but not limited to cigarette smoke, which may influence the magnitude and direction of the response.

Current measures of antioxidant status in an individual vary greatly among different research settings, making comparison of results problematic. The establishment of "gold standard" biomarkers of oxidative stress and establishment of guidelines to accurately and precisely determine levels of a given marker in normal, healthy individuals should help to resolve the uncertainties in the literature. Better measurements may also help to determine why a diet high in fruits and vegetables is often more beneficial than specific antioxidant supplements. Additional research focusing on critical events that contribute to disease progression will help in defining the most efficient times for intervention with antioxidant supplementation.

Future research must focus on defining molecular mechanisms that engender oxidative stress, transforming healthy conditions to diseased states, and identifying timelines for effective interventions with antioxidants as either preventive or therapeutic agents. Although much of the current literature about antioxidants, including administration of pharmacological or dietary amounts, focuses on enhancing ROS elimination and inhibiting ROS generation, many other cellular processes are likely involved. An improved understanding of these cellular processes and how they relate to measures of oxidative stress will provide important clues about those who will benefit most or be placed at risk from antioxidant usage, whether provided as foods or supplements. The identification of the reliable and sensitive biomarkers of antioxidant exposures, of their biological effects or consequences, and of susceptibility factors including genetic and environmental modifiers will have far-reaching implications for the monitoring and treatment of oxidative stress related to health and disease conditions.

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Chapter 17

Choline and Brain Development

Mihai D. Niculescu
Advanced Nutrigenomics LLC, Concord, NC, United States

I INTRODUCTION

An increasing amount of evidence supports the hypothesis that chronic illness in adult life has, in part, its origins before birth due to various environmental exposures [1]. In such cases, prevention rather than treatment becomes the active principle in establishing long-term public health policies that will enable an overall improvement in the health status of the general population [2]. Various nutrient deficiencies (omega-3/6 fatty acids, iron, protein/amino acids, energy restriction, folate, choline, etc.), occurring during pregnancy or perinatally, have been associated with defects in brain development that range from impaired physiological functions such as decreased visual acuity to severe birth defects [3–9]. Moreover, such early alterations in brain development have been associated, in many cases, with long-term functional alterations of brain functions, within adulthood and the aging period [10–12].

The importance of adequate nutrition during brain development has been repeatedly reinforced by many studies. A growing body of evidence indicates that the relationship between various nutrients and the development of the nervous system is complex and not necessarily confined to one specific period of gestation, but there is no doubt that specific nutrients play essential roles in neural development [10,13,14]. This chapter discusses the role that choline and its metabolite betaine have in brain development and also the subsequent implications in the physiology of memory and brain aging.

II CHOLINE METABOLISM AND BIOCHEMISTRY

A Intestinal Absorption

Dietary free choline or choline-containing esters (e.g., phosphatidylcholine, PC) are first hydrolyzed in the intestine. The free choline is oxidized, in part, by the gut bacteria to betaine (Fig. 17.1) or further metabolized to methylamines [15], whereas the choline-containing esters are hydrolyzed by enzymes from the pancreatic secretions and from intestinal mucosal cells, such as phospholipases A_1, A_2, and B [16]. The remaining free choline is absorbed by the enterocytes via carrier-mediated transport [17,18], whereas betaine is absorbed most probably via active Na^+ or Cl^--coupled, and also via passive Na^+-independent transport systems (reviewed in [19]), at a faster rate than choline [17,18]. The bioavailability of choline-containing compounds is different in infants than in adults, probably as a consequence of differences in both the physiology of their digestive system [16] and the choline content of the milk [20].

B Transport and Tissue Uptake

Once absorbed into enterocytes, choline is transported to the liver via the portal circulation mainly as PC [21], or it is incorporated into chylomicrons and released into the systemic circulation via the lymphatic system [22,23]. Choline accumulates in all tissues [24] by diffusion and mediated transport (reviewed in [25,26]) using three distinct uptake systems: low-affinity facilitated diffusion, high-affinity Na^+-dependent transport, and an Na^+-independent transport with intermediate affinity [25,26]. Based on the affinity for choline, three types of transporters have been identified: cation transporters with low affinity, choline-transporter-like with intermediate affinity, and choline transporters (CHTs) with high affinity [25,26]. In addition, PC is trafficked via the ATP-binding cassette transporters (especially ABCA1 and ABCG1) [27–29]. In liver, choline uptake is also

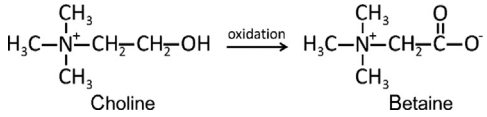

FIGURE 17.1 Molecular structures of choline and betaine.

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dependent on the remodeling of HDL-PC by secretory phospholipase A_2 [30]. Choline is transported across the blood–brain barrier by the CHT high-affinity system, represented by the solute carrier family 5 (CHT), member 7 (SLC5A7, known also as CHT or CHT1) [31–34]. Within the brain cells, this system depends partially on the integrity of endosomal apparatus [35], and it supplies choline for acetylcholine synthesis in presynaptic neurons [36]. Preliminary studies suggested that the CHT system could be enhanced by treatment with cytidine-5′-diphosphocholine (CDP-choline), specifically within cognitive areas such as the frontal cortex [37]. The CDP-choline pathway is the main source of endogenous PC synthesis in brain, and it contributes to axon formation, growth, and branching of primary sympathetic neurons [38]. Preliminary studies suggested that CHT trafficking to cell membrane (and the consequent increase in choline uptake) may be under the transient control of protein kinase C activation [39].

Choline is excreted in the primary urine by glomerular filtration, but only 2% of the filtered choline is found in the final urine because of the intense reabsorption present mainly in the proximal tubules [40], operated by an organic cation transport system [41].

C Metabolism

Fig. 17.2 presents a general overview of choline metabolism. Of special importance is the accumulation of choline by liver, kidney, brain, mammary gland, and placenta [24,25,42]. Choline is involved in three major pathways: acetylcholine synthesis, methyl donation via its oxidation to betaine, and PC synthesis. The latter two have a special importance in brain development [43,44]. PC synthesis occurs by two independent pathways (Fig. 17.2). Choline is phosphorylated by choline kinase and converted subsequently to CDP-choline. In combination with diacylglycerol, CDP-choline forms PC (reaction catalyzed by diacylglycerol cholinephosphotransferase). In an alternate pathway, choline is synthesized de novo by the methylation of phosphatidylethanolamine (PtdEtn) to PC in a reaction catalyzed by PtdEtn-*N*-methyltransferase (Pemt, EC 2.1.1.17) using *S*-adenosylmethionine as methyl donor [45,46]. Although most active in liver, this pathway is also active in other tissues, such as fetal brain and mammary gland [47–49]. In brain, choline regulates the Na^+, K^+-ATPase activity, although its mechanism of action is not yet completely understood [50].

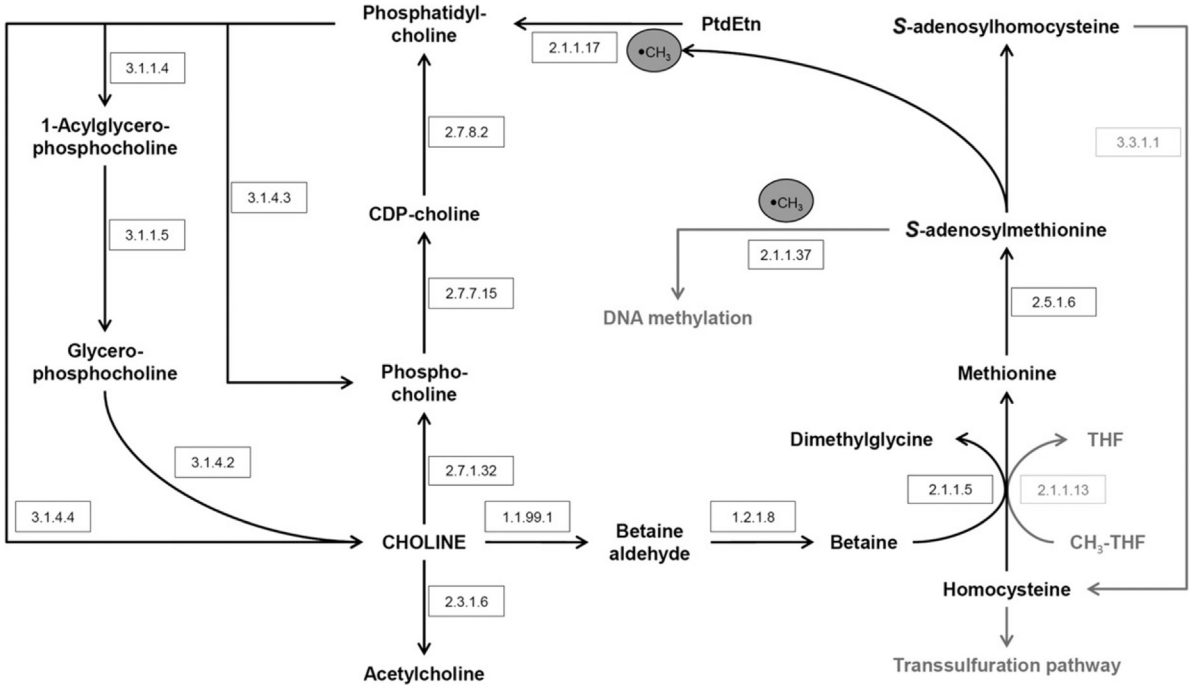

FIGURE 17.2 Choline metabolism. Enzymes are represented by EC numbers (1.1.99.1, choline dehydrogenase; 1.2.1.8, betaine-aldehyde dehydrogenase; 2.1.1.5, betaine-homocysteine *S*-methyltransferase; 2.1.1.13, methionine synthase; 2.1.1.17, PtdEtn *N*-methyltransferase; 2.1.1.37, DNA (cytosine-5-)-methyltransferase; 2.3.1.6, choline *O*-acetyltransferase; 2.5.1.6, methionine adenosyltransferase; 2.7.1.32, choline kinase; 2.7.7.15, choline-phosphate cytidylyltransferase; 2.7.8.2, diacylglycerol cholinephosphotransferase; 3.1.1.4, phospholipase A2; 3.1.1.5, lysophospholipase; 3.1.4.2, glycerophosphocholine phosphodiesterase; 3.1.4.3, phospholipase C; 3.1.4.4, phospholipase D; 3.3.1.1, adenosylhomocysteinase). PtdEtn, phosphatidylethanolamine; THF, tetrahydrofolate.

Choline is also involved in the one-carbon metabolism via its irreversible oxidation to betaine [51], which methylates homocysteine to form methionine, thus contributing to the *S*-adenosylmethionine synthesis and linking choline with folate metabolism (Fig. 17.2). Note that choline and folate metabolisms interact dynamically. Relative to brain, folate-deficient intakes or *Mthfr* heterozygosity enhanced the conversion of choline to betaine in order to sustain the homeostasis of methyl groups available for homocysteine methylation [52]. When rats were exposed to a folate-deficient diet for 10 weeks, choline levels decreased in liver, lung, kidneys, and heart [53]. Surprisingly, choline levels were moderately elevated in the cortex and striatum [53].

During pregnancy, choline is transported across the placenta by active mechanisms, against a concentration gradient [54]. The fetus is exposed to a high concentration of choline, and plasma choline concentration progressively declines after the first weeks of life [55]. In human newborns, plasma-free choline can reach concentrations of approximately 70 μM [56], whereas in adults, plasma-free choline is much lower (7−20 μM) [56,57]. The majority of choline in blood circulates as PC (1−2.5 mM) [56,57].

TABLE 17.1 Adequate Intakes for Choline

Group	Age	Adequate Intake (mg/day)
Infants and children	0−6 months	125
	7−12 months	150
	1−3 years	200
	4−8 years	250
	9−13 years	375
Boys	14−18 years	550
Girls	14−18 years	400
Pregnant women	All ages	450
Lactating women	All ages	550
Other men		550
Other women		425

Source: Adapted from Institute of Medicine and National Academy of Sciences USA, Choline, in: Dietary Reference Intakes for Folate, Thiamin, Riboflavin, Niacin, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline, National Academy Press, Washington, DC, 1998, pp. 390−422.

III CHOLINE IN FOODS AND DIETARY REQUIREMENTS

A Dietary Sources

Most of the foods we eat contain various amounts of free choline, choline esters, and betaine [48]. In 2004, the U.S. Department of Agriculture (USDA) released its first database on choline content in common foods (https://www.ars.usda.gov/ARSUserFiles/80400525/Data/Choline/Choln02.pdf). In most foods, free choline and PC are the most abundant compounds. The foods most abundant in choline are of animal origin, especially eggs and liver, but some vegetables also have significant amounts of free choline and PC (Brussels sprouts, cauliflower, nuts, etc.). Cereals and many baked products contain high amounts of betaine as well.

In human breast milk, free choline and all main choline esters are abundant, with total choline levels between 0.6 and 2 mM [58]. Manufacturers of infant formulas have modified the content of choline compounds to levels similar to those of human breast milk [20,58].

B Dietary Requirements

Although choline was not initially considered an essential nutrient because of its endogenous de novo synthesis from PtdEtn [59], human studies during the past two decades have demonstrated that dietary choline is required (reviewed in [60]). In 1998, the U.S. Institute of Medicine (Food and Nutrition Board) established for the first time adequate intake (AI) and tolerable upper intake limit (UL) values for choline based on limited human studies [61] (Table 17.1). These values remained unchanged after the 2010 revision of the Dietary Reference Intakes by the USDA. The UL values range from 1000 mg/day in children to 3500 mg/day in adults [61]. However, for some age categories for which adequate data were missing, AI values have been set by extrapolating from adult values (for ages 1−18 years) and for infants (for ages 7−12 months) [61]. Although a previous controlled study indicated that choline intakes may be adequate [62], a later epidemiological study using participants from the Framingham Offspring Study indicated that the mean intake for total choline (energy adjusted) is below the AI values, with a mean intake of 313 mg/day; moreover, there was an inverse association between choline intake and plasma total homocysteine concentration in subjects with low-folate intakes [63]. This study strengthened previous similar findings in pregnant women, where a significant percentage of individuals had lower choline intakes than the recommended AI values [64,65]. The realization that choline intakes in the general U.S. population may not be adequate has been refined by studies indicating that high-frequency genetic variations within genes involved in choline and folate metabolism can significantly alter the threshold for dietary requirements, thus raising the issue of individual-specific AIs for choline, rather than the general recommendations that are in place today (Table 17.1) [60,66−69]. Age- and gender-specific

differences have also been reported for choline concentrations in plasma and human brain, indicating that the definition of dietary choline intakes should also take into consideration the physiological variations associated with gender and age [70,71].

C Interactions With Other Nutrients and Environment-Related Chemicals

Several animal studies have indicated that choline metabolism can be modulated by interaction with other nutrients and vitamins or by chemicals present in foods, water, food packaging, or food containers.

Dietary choline supplementation has proved beneficial against physical, neurological, and behavioral alterations induced by alcohol exposure. When pregnant rats were subjected to both alcohol and choline supplementation, behavioral measures in the offspring were improved compared to those of a group exposed only to alcohol, indicating that early choline supplementation could reduce the severity of fetal alcohol outcomes [72]. Within the same model, alterations induced by alcohol exposure in working memory were mitigated by choline supplementation [73]. Rats exposed postnatally to alcohol, and later choline supplemented, had significant reductions in the severity of trace eyeblink deficits induced by alcohol exposure [74].

The exposure to nicotine has been reported to adversely affect choline metabolism and brain development, although not all reported results are convergent. Gestational exposure of rats to nicotine decreased the choline uptake in the brains from offspring, due to lower binding to the high-affinity transporter, whereas changes in the acetylcholine esterase and choline acetyltransferase (ChAT) levels were variable and brain-area specific [75]. When young mice were exposed to cigarette smoke (containing a high and a low nicotine dose), those exposed to high nicotine smoke had increased choline uptake in the hippocampus and cerebral cortex compared to those exposed to low nicotine smoke or controls [76].

In rats, choline supplementation reversed the long-term alterations in learning and memory induced by Pb^{2+} exposure, which associated with higher gene and protein expression for the *N*-methyl-D-aspartate (NMDA) receptor subunit 1 (NR1), compared to the group exposed only to Pb^{2+} [77].

Mice fed on a choline-deficient diet registered increased nonheme iron in the liver. The assessment of protein and mRNA expression of genes involved in iron metabolism and transport suggested that dietary choline deficiency may be associated with enhanced iron intake and reduced hepatic iron efflux [78].

Choline availability is enhanced by supplementation with vitamins B_6 and B_{12}, which are involved in folate and homocysteine metabolism (Fig. 17.2) [79]. However, it is not clear whether these interactions have consequences for brain development.

Choline metabolism is also altered by the presence of other chemicals that, although not nutrients, can be found in packaging materials, drinking water, cosmetics, or other plastics used for foods. Examples are bisphenol A, diethanolamine, and arsenic [80–85].

D Consequences of Dietary Choline Deficiency in Humans

Studies performed in animals and humans have categorized the effects induced by dietary choline deficiency into three groups: (1) changes in acetylcholine synthesis and release in brain, (2) organ dysfunction/metabolic changes in adults eating a low-choline diet for a relatively short time, and (3) birth defects (abnormal neural tube closure and cleft palate) in newborns from mothers eating diets lower in choline content, as well as effects of choline on neural-related cell proliferation and survival in rodents.

Historically, the first reports on the effects induced by changing dietary choline focused on acetylcholine release in human brain and the consequences this had on memory, small motor movements, and the release of other neurotransmitters such as 7-aminobutyric acid (reviewed in [86]). In this context, the effectiveness of choline supplementation in improving the acetylcholine release was, for most of the studies, limited to individuals with neurological disorders such as tardive dyskinesia and Alzheimer's disease [87]. Using rodent models, dietary choline has been shown to modulate the density of the benzodiazepine receptors and the function of the GABAergic receptors in the cortex [86], but whether these findings can be applied to healthy humans remains unclear.

Human dietary requirements for choline have been studied in subjects fed low-choline diets under controlled conditions; they developed reversible fatty liver as well as liver and muscle damage [88,89]. These clinical outcomes were associated with increased apoptosis in lymphocytes [90]. Premenopausal women were less likely to develop organ dysfunction when fed a low-choline diet than were men or postmenopausal women, most likely because estrogen contributes to the activation of the gene that regulates the endogenous synthesis of choline (PtdEtn-*N*-methyltransferase gene (*PEMT*)) [91]. Interestingly, the risk of developing such clinical signs was associated with the presence of several polymorphisms within genes involved in folate and choline metabolism, such as the *PEMT* gene, the choline dehydrogenase gene (*CHDH*), and the 5,10-methylenetetrahydrofolate dehydrogenase (*MTHFD1*) gene, suggesting that dietary choline requirements may differ with genotype, gender, and estrogen status [68,92].

The third category of effects relates to critical periods when choline is needed for fetal and infant development, for which most studies have focused on neural and brain development.

IV CHOLINE AND NEURAL DEVELOPMENT

Dietary choline is essential in at least two distinct stages of brain development: neurulation (formation and closure of the neural tube) and a later stage (late pregnancy and potentially the neonatal period) during the maturation of hippocampus and other brain areas. Although the mechanisms responsible for these outcomes have been partially elucidated in animal models, confirmation in human studies is available only for early pregnancy.

A Choline Availability in Early Pregnancy

When gestation day 9 mouse embryos were exposed in vitro to either an inhibitor of choline uptake (2-dimethylaminoethanol) or an inhibitor of PC synthesis (1-*O*-octadecyl-2-*O*-methyl-rac-glycero-3-phosphocholine; ET-18-OCH3), they developed craniofacial hypoplasia and open neural tube defects in the forebrain, midbrain, and hindbrain regions [93]—alterations that are similar to those induced by folate deficiency [94]. Increased cell death was associated especially with the inhibition of PC synthesis [93]. A subsequent study using the same mouse embryo model revealed that the mechanisms responsible for these outcomes were related to alterations in choline metabolism pathways [95], stressing the importance of dietary choline. These two animal studies were enhanced by data from human epidemiological studies describing similar outcomes in newborns from mothers who ate diets low in choline during pregnancy. In these studies, Shaw et al. reported that maternal dietary choline and betaine intakes during pregnancy inversely correlated with the risk of having a baby with neural tube defects and orofacial clefts [64,65]. Women in the lowest quartile of total choline intake during the periconceptional period (290.41 mg/day) and eating diets low in folate content had a fourfold higher risk of having a baby with neural tube defects (spina bifida and anencephaly) than the women in the highest quartile for choline intake and with a low-folate diet [65]. A similar relationship was also found between maternal choline intake and the increased risk of cleft lip with cleft palate [64]. However, a recent study by the same group failed to replicate these findings [96]. It is not clear whether this discrepancy was due to differences in study design or differences in the recruited populations [96]. Another study performed by the same team found a surprising association between higher intakes of choline and increased risk for metopic synostosis [97].

Independent of the maternal choline intake, the presence of single nucleotide polymorphisms of two genes involved in choline metabolism—choline kinase A (*CHKA*) and CTP:phosphocholine cytidylyltransferase 1 (*PCYT1A*)—increased the risk of spina bifida in infants [98]. Together, these data suggest that the risk for neural tube defects may be related to the maternal dietary choline and folate intakes and to the presence of genetic polymorphisms in genes related to the metabolism of these two nutrients in the offspring.

B Choline Availability in Late Pregnancy

During later gestation, rat and mouse models have been used to explore the role of choline in fetal brain development.

1 Choline Deficiency Inhibits Cell Proliferation

Rats and mice fed a low-choline diet in late pregnancy (gestational days 12−17 in mice and days 12−18 or 20 in rats) had reduced neural precursor cell proliferation and increased apoptosis in fetal hippocampus and cortex [99−101]. Similar outcomes were reported when pregnant mice were fed a low-folate diet [102], again suggesting the potential synergistic mechanisms of action between folate and choline. These alterations were confirmed in cell culture studies using primary neurons, pheochromocytoma cells, and human neuroblastoma cells. Dividing neuron-like cells (PC 12) exposed to a choline-deficient medium had decreased cell division and increased apoptosis and had diminished concentrations of PC in their membranes [99,103]. The induced apoptosis is caspase 3-dependent in both cell culture and fetal brain models [101,103]. These changes were associated with important alterations of proteins involved in cell signaling, neuronal differentiation, and the regulation of cell-cycle progression. Choline deficiency altered the expression of structural and signaling proteins such as TGF-β_1, vimentin, and MAP1 in the rat hippocampus [104]. Decreased cell proliferation was also associated with increased expression of neuronal and glial differentiation markers vimentin, TOAD-64 (dihydropyriminidase-like 2; Dpysl2), and calretinin [100,104,105]. Moreover, choline deficiency altered the protein expression of netrin and DCC, two proteins required for axonal growth and guidance in the developing nervous system [106]. These data suggested that choline deficiency induces a net trend toward the differentiation of neural progenitors and a subsequent reduction of the pool of the available precursor cells. Some differences persist for the lifetime of the offspring of treated mothers; prenatal choline supplementation decreased calretinin protein levels in the adult (24-month) mouse hippocampus [105].

A study revealed that the alterations in cell proliferations induced by fetal choline deficiency are not confined to neural cells. Angiogenesis in mouse fetal hippocampus was inhibited by choline deficiency (gestational day 17) due to decreased proliferation of endothelial cells [107]. The reduction was associated with increased expression of genes involved in angiogenic signaling (*Vegfc* and *Angpt2*) [107].

Regarding the link between choline and folate metabolisms (discussed previously), limited studies indicated that choline supplementation could partially offset the deleterious effects of folate deficiency on fetal brain development. Whereas folate deficiency during late pregnancy (gestational days 11−17) presented less cell proliferation and increased apoptosis within septum, hippocampus, striatum, and the anterior and midposterior neocortex, the concomitant exposure to a folate-deficient, choline-supplemented diet partially mitigated these outcomes in some of the brain areas but not all [108].

The mechanisms associating choline deficiency with decreased cell proliferation are, in part, related to the overexpression of cyclin-dependent kinase inhibitors p27Kip1 [109], p15Ink4b [109,110], and Cdkn3 [110,111], indicating that choline deficiency inhibits cell proliferation by inducing G_1 arrest because of the inhibition of the interaction between cyclin-dependent kinases and cyclins. Moreover, in human neuroblastoma cells (IMR-32), choline deficiency decreased the phosphorylation of the retinoblastoma protein (p110, Rb) [111]. This interaction model between p27Kip1 and p15Ink4b cyclin-dependent kinase inhibitors, TGF-β, and the Rb proteins fits the previously described model of cell-cycle regulation (reviewed in [112]), in which the net outcome is cell-cycle arrest in the G_1 phase of the cell cycle. These findings were reinforced by a study using mouse hippocampal and cortical progenitor cells exposed to choline deficiency for 48 hours. Using oligonucleotide arrays, the authors reported extensive changes in more than 1000 genes, of which 331 were related to cell division, apoptosis, neuronal and glial differentiation, methyl metabolism, and calcium-binding protein ontology classes [113], where, again, the net result was toward reduced cell proliferation, increased apoptosis, and increased neuronal and glial differentiation.

2 Choline Deficiency Alters Gene Expression via Epigenetic Mechanisms

Epigenetic mechanisms consist of chemical changes of the chromatin (DNA and histones) that do not alter the DNA sequence and that establish meiotically and mitotically stable, heritable patterns of gene expression [114,115]. These mechanisms consist of DNA methylation and hydroxymethylation, histone modifications, along with the modulation of epigenetic marks by noncoding RNA species [14,115,116].

a Epigenetic Mechanisms Regulate Gene Expression

DNA methylation is represented by the substitution of hydrogen with methyl groups to DNA (reviewed in [117]). In mammalians, the majority of DNA methylation occurs at carbon 5 of the cytosine ring (5-methylcytosine; 5mC) only when the cytosine is followed by a guanine nucleotide (CpG site), but methyl groups can be also added to other nucleotides [117]. This process is catalyzed by DNA methyltransferases (DNMTs). During the S-phase of cell-cycle progression, the DNA methylation status of the parental DNA strand is duplicated on the newly synthesized DNA by maintenance DNA methyltransferase, DNMT1 [118]. However, DNA methylation can also occur at previously unmethylated CpG sites (de novo DNA methylation), catalyzed by de novo DNA methyltransferases, DNMT3a and 3b, and with participation of DNMT2 and 3L [118].

When DNA methylation occurs within promoter regions, it usually associates with gene underexpression and chromatin compaction [51], but instances have been described in which promoter hypermethylation prevented the binding of inhibitory factors, thus allowing for promoter activation and gene overexpression [119]. The establishment of cell type-specific DNA methylation patterns contributes decisively to shaping the cellular phenotypes of differentiated cells [118].

Within the concept of epigenetic regulation of gene expression, an important feature is genomic imprinting, allowing genes to be expressed in a parent-of-origin manner (imprinted genes), and this process being the molecular basis for monoallelic expression [120]. During early embryogenesis, most of the parental DNA methylation patterns are erased by active and passive demethylation mechanisms (with the exception of some imprinted regions), whereas new DNA methylation patterns are established by de novo methylation. The establishment of new epigenetic patterns continues during fetal morphogenesis and in the early postnatal period [121].

The epigenetics of DNA also includes the hydroxylation of methyl groups attached to cytosine, as an intermediary step in active DNA demethylation, with important functional consequences on gene activation [122]. This groundbreaking discovery provided the first plausible mechanism for the previously observed active DNA demethylation [116].

Chromatin modifications occur at the flexible tail regions of histones. These modifications include, but are not limited to, methylation, acetylation, phosphorylation, ubiquitination, and ADP ribosylation [123]. In concert with DNA methylation, histone modifications allow for

the reversible switch between chromatin relaxation and compaction and also the establishment of the degree of access that transcription factors have to promoter regions [118,123]. Examples are methylation of histone H3 at its lysine 9 and 27 residues (K9 and K27), allowing for chromatin compaction and inhibition of gene expression, and trimethylation of H3K4 that induces transcriptional activation and promoter activation [123].

MicroRNAs (miRNAs) are noncoding RNA species, up to 25 nucleotides in length, that contribute to gene expression regulation through RNA interference [124]. Their epigenetic role consists of the modulation of expression for several genes involved in the epigenetic machinery, which are responsible for DNA and histone modifications (e.g., *DNMT3a/b*, *HDAC1/4*, and *MeCP2*) [124]. Some genes encoding miRNA species can also be epigenetically regulated because their gene expression is highly dependent on their promoter methylation (reviewed in [124]).

b DNA Methylation, Fetal Development, and Cell Differentiation

DNA methylation is very important during embryogenesis and late fetal development. Although the original DNA methylation pattern is germline specific (sperm DNA is hypermethylated compared to oocyte DNA), almost immediately after fertilization (within one or two cell divisions) there is a dramatic erasure of methylation, which continues until blastocyst implantation [121]. Following implantation, mouse embryonic stem cells are subjected to de novo methylation catalyzed by *Dnmt3a* and *Dnmt3b* genes (de novo methylases), with the exception of certain tissue-specific genes that remain unmethylated [121]. Once established, the new methylation pattern is conserved during cell replication (catalyzed by Dnmt1 in the S-phase of cell division [125]). These methylation patterns can be changed as cells differentiate (cell differentiation is associated with a genome-wide DNA hypomethylation followed by remethylation [117]) and can be altered by dietary intake of methyl donors (discussed next).

c DNA Methylation and Neural Development

Neural development is influenced by DNA methylation. Overall levels of methylation decrease as neuronal differentiation proceeds [126], and the treatment of neural precursor cells with demethylating agents induces them to differentiate into cholinergic and adrenergic neurons [127]. These methylation patterns are cell type-specific: Whereas mature neurons express DNMTs, these genes have much lower levels of expression in oligodendrocytes and astrocytes in the white matter [128]. The expression of DNMTs has a different importance, based on the differentiation stage of the cell: deletion of Dnmt1 in postmitotic neurons does not affect overall levels of DNA methylation, whereas the same deletion in neural progenitors markedly decreases methylation levels and causes severe defects in neurogenesis [129]. Astrocyte differentiation is also dependent on the methylation status of glial fibrillary acidic protein (*Gfap*) promoter at the binding site of STAT3 transcription factor. This promoter site becomes hypomethylated before differentiation, and its methylation prevents gene activation by STAT3 and astrocyte differentiation in fetal brain [130].

d Choline Deficiency Alters Epigenetic Status in Fetal Brain

Although the relationship between nutrition and epigenetics has been firmly established [131], less is known about the epigenetic mechanisms involved in nutritionally triggered alterations in fetal brain development. However, available data allow us to identify an important role for choline in DNA methylation during brain development (reviewed in [132]). Maternal choline deficiency decreased the global DNA methylation in the neuroepithelial layer of the fetal hippocampus [110], whereas opposite effects were reported in the fetal brains from $Pemt^{-/-}$ mice, which also had increased *S*-adenosylmethionine levels [133]. Interestingly, $Pemt^{-/-}$ mice also had altered methylation of the lysine 4 and 9 residues within histone 3, suggesting that alterations in choline availability to the fetal brain may be crucial for both DNA and histone methylation [133]. Along with decreased global methylation, changes in gene-specific methylation were reported, where the promoter of cyclin-dependent kinase 3 (*Cdkn3*) was hypomethylated by choline deficiency in the progenitor layer of the hippocampus and in human neuroblastoma cells [110,111]. These alterations were associated with increased protein expression of this cyclin-dependent kinase inhibitor [110], and this model is consistent with previous findings regarding the epigenetic regulation of cyclin-dependent kinase inhibitors and their roles in cell proliferation [134]. Choline deficiency also induced DNA hypermethylation of the calbindin 1 promoter on the mouse fetal hippocampus, which associated with region-specific hypomethylation of monomethyl-lysine 9 of histone 3 (H3K9me1) and dimethyl-lysine of histone 3 (H3K9me2) in the hippocampus [119].

The epigenetic roles of choline are not confined to neural cells. Within the fetal hippocampus, the reduction in angiogenesis induced by choline deficiency also involved the DNA hypomethylation of CpG islands within the *Vegfc* and *Angpt2* promoters, two genes that are implicated in angiogenic signaling [107].

Because dietary choline is important in the maintenance of the *S*-adenosylmethionine pool (the methyl donor for DNA methylation), along with folate and methionine (Fig. 17.2), it is attractive to hypothesize that

choline, by influencing the epigenetic status of the developing brain, could thereby induce permanent epigenetic changes associated with alterations of brain function at later ages [14]. Fig. 17.3 summarizes the hypothesized role that choline may play in the regulation of early brain development.

V LONG-LASTING CONSEQUENCES OF PRENATAL CHOLINE AVAILABILITY

A Molecular and Functional Changes

The changes induced by dietary choline in fetal brain have long-lasting effects that alter the neuronal function throughout the adult life. When pregnant rats were choline supplemented during late gestation, basal and receptor-stimulated phospholipase D activity was upregulated in the hippocampus of the offspring during postnatal development [135]. Acetylcholine metabolism and choline uptake mechanisms were also permanently altered in the adult brain. ChAT and acetylcholinesterase (AChE) activities were increased in the adult hippocampus of rats exposed to choline deficiency while in utero, and choline incorporation into acetylcholine was more dependent on high-affinity choline uptake mechanisms, compared to controls or to rats that were choline supplemented while in utero [136]. The increase in AChE activity was later found to be due to increased AChE protein synthesis [137], strongly suggesting that gene expression or post-transcriptional regulation was permanently altered by choline deficiency. This hypothesis was confirmed later in a study showing that the gene expression of the choline transporter, Cht, was increased in the adult hippocampus from rats exposed to prenatal choline deficiency, and that this correlated with an increased number of neurons that were CHT immunoreactive [138]. In addition, many other changes in gene expression were described to occur in the adult hippocampus and cortex, initiated by prenatal choline manipulation [139].

Cell signaling is also influenced by prenatal choline availability. In juvenile rats, the phosphorylation and activation of hippocampal mitogen-activated protein kinase and cAMP response element binding protein in response to glutamate, NMDA stimulation, or depolarizing concentrations of K^+ were increased by choline supplementation and reduced by choline deficiency while in utero [140]. Choline supplementation while in utero also increased the levels of nerve growth factor (NGF) in the adult rat hippocampus and cortex [141], suggesting that prenatal choline availability has an important role in promoting neurogenesis in the adult hippocampus, which is mediated by the nootropic action of NGF [142]. Opposite changes were reported for choline deficiency while in utero in other areas of the forebrain, such as medial septal nucleus, nucleus of the diagonal band, and the nucleus basalis of Meynert [143].

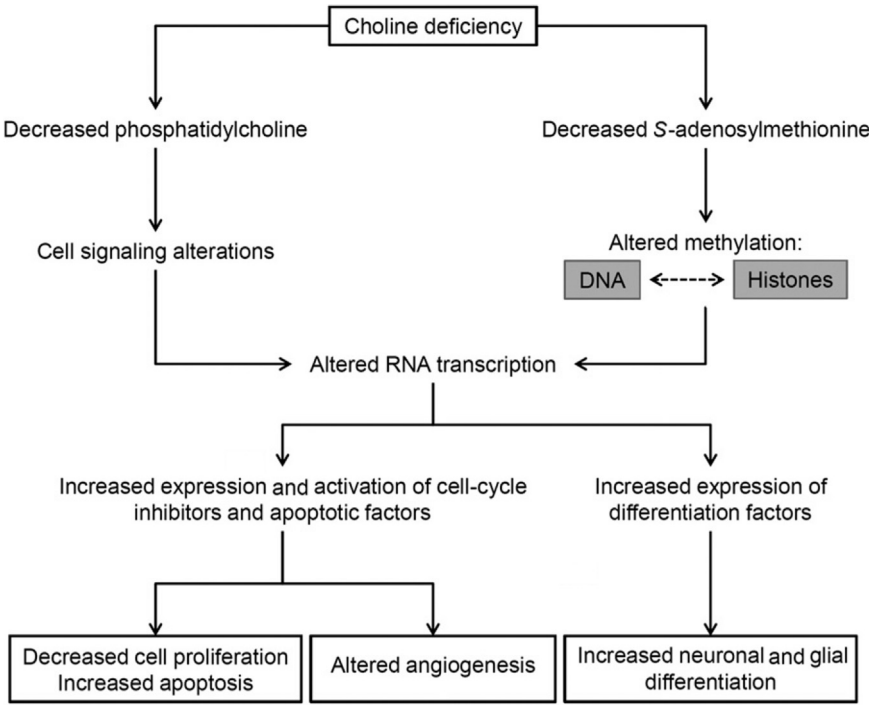

FIGURE 17.3 Choline deficiency alters brain development.

Although confirmation is needed in humans, these findings provided evidence that prenatal choline availability initiates a pattern of permanent metabolic alterations (metabolic imprinting) that, once established, plays an important role in later life [144].

B Choline Deficiency Induces Cognitive and Memory Deficits

The functional and molecular changes previously described are, at least in part, responsible for behavioral and memory changes initiated by prenatal variation in the availability of choline to developing brain (reviewed in [43]). Prenatal choline supplementation protects against the neurotoxicity induced by the administration of the NMDA receptor antagonist dizocilpine (MK-801) to female adolescent rats [145,146]. When status epilepticus was induced in adult rats using kainic acid, rats receiving supplemental choline between gestational day 11 and postnatal day 7 performed better in the water maze tests than did the deficient and control groups, whereas the hippocampal ChAT activity was 18% lower in the choline-deficient animals compared with the other two groups [147]. However, prenatal choline-deficient rats were not more susceptible to seizure induction by kainic acid than the group receiving adequate choline during fetal development [148].

Maternal dietary choline availability during late pregnancy was associated with long-lasting changes in the hippocampal function of the adult offspring. Choline supplementation during this period enhanced visuospatial and auditory memory in the adult rats throughout their life span [149–153]. It also enhanced a property of the hippocampus, long-term potentiation [154–156]. The offspring from mothers fed a choline-deficient diet manifested opposite outcomes [150,154].

In men, the choline concentration in the anterior cingulated cortex correlated with age, and higher total choline values were positively correlated with faster performance on the Stroop Interference task [157]. Postmortem assessment of choline levels in the brains of men with Alzheimer's disease indicated lower levels of plasmalogen choline in the prefrontal cortex than in controls, but it was unclear whether there was a mechanistic association between choline levels and disease because other components within phospholipids were also altered (i.e., docosahexaenoic acid) [158].

VI IMPLICATIONS FOR HUMAN BRAIN DEVELOPMENT

It is difficult to extrapolate to humans the findings reported using animal models. However, data are available to support the hypothesis that similar mechanisms are involved in humans. Because of ethical constraints, no studies are available in children or pregnant mothers to validate the rodent model. Because pregnant women are at risk of becoming choline deficient [60,159–161], and possibly having increased risk of giving birth to infants with neural tube defects [65], the recommendation that pregnant women should attempt to consume diets adequate in choline content seems reasonable. In addition, because half of the population has gene polymorphisms that affect choline and folate metabolisms [68,92], it is likely that different individuals may have different dietary requirements for choline and may need to pay special attention to choline intake not only during pregnancy but also during all other life stages.

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Chapter 18

Dietary Phytochemicals in Neurodegenerative Disease

Aurélie de Rus Jacquet[1,2], Mitali A. Tambe[1,3] and Jean-Christophe Rochet[1]

[1]*Purdue University, West Lafayette, IN, United States,* [2]*Howard Hughes Medical Institute, Seattle, WA, United States*

[3]*Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, United States*

I INTRODUCTION

Over the past century there has been a significant increase in human life expectancy in developed countries, a phenomenon that is largely attributed to medical advances that have led to a decrease in mortality from infectious diseases and cardiovascular disease during this period [1]. However, a downside to this trend has been an increase in the incidence of age-related neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). AD is the most common neurodegenerative disorder, affecting approximately 36 million people worldwide, including 11% and 32% of individuals over the age of 65 years and 85 years, respectively [2,3]. PD is the second most common neurodegenerative disease, affecting 5 million people worldwide including 5% of the population over the age of 85 years [4–6]. Not only do these devastating diseases have a profound emotional impact on patients and their families, but they also impose a major economic burden on society. For example, the cost of AD worldwide is estimated to have exceeded $800 billion in 2015 [7], and in 2010 the cost of PD is reported to have been greater than $14 billion in the United States alone [8]. These costs are expected to increase to unsustainable levels as our population continues to age over the next few decades.

A major limitation of current approaches to treating AD or PD patients is that no disease-altering therapies are available to slow the underlying neurodegeneration. Instead, the options available to patients consist of symptomatic therapies that provide only marginal benefits in the case of AD patients, or a real but temporary relief of motor symptoms in the case of PD patients. As a result, research in the AD and PD field is largely focused on elucidating the molecular underpinnings of these disorders to enable the development of disease-altering therapies. A substantial part of this effort has been aimed at characterizing a role for the diet in AD and PD, with a view towards identifying dietary components that reduce the risk of these diseases or that slow the underlying neurodegeneration in the brains of patients. In particular, dietary plant constituents including polyphenols have been examined extensively as potential neuroprotective agents based on epidemiological evidence suggesting that their consumption may lead to a reduced risk of AD or PD [9–17].

In this chapter, we provide an overview of dietary phytochemicals that have shown promise as potential neuroprotective agents in AD and PD. We begin with a summary of the symptoms, pathobiology, treatments, and underlying molecular deficits of both diseases. Next, we summarize neuroprotective effects of plant constituents in AD and PD models, with an emphasis on the effects of polyphenols, and we discuss molecular mechanisms by which these agents are thought to alleviate neurotoxicity in AD and PD models. We conclude by looking ahead to future research that could set the stage for using phytochemicals as neuroprotective agents in the treatment of AD or PD.

II ALZHEIMER'S DISEASE

A Symptoms, Neuropathology, and Treatments

Characteristic symptoms of AD include: memory loss that interferes with daily activities; an impaired ability to

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learn, reason, or solve problems; confusion; changes in mood or personality; and anxiety, agitation, and sleep disturbances [3]. These symptoms are largely attributed to an array of pathological changes in the brain, including the presence of extracellular amyloid plaques (also referred to as neuritic or senile plaques) and intracellular neurofibrillary tangles (NFTs) [2,18,19]. Amyloid plaques are enriched with fibrillar forms of amyloid-β (Aβ), a peptide of 38−43 amino acid residues that is excised from a larger transmembrane protein, the amyloid precursor protein (APP), via sequential proteolytic cleavages catalyzed by β-secretase (BACE) and the γ-secretase complex (Fig. 18.1). $A\beta_{42}$, the 42-residue form of Aβ, has an enhanced propensity to form amyloid fibrils compared to the more abundant $A\beta_{40}$ peptide and is the predominant Aβ species found in amyloid plaques [19,20]. NFTs are enriched with fibrillar forms of the microtubule-binding protein tau [2,18,19]. AD neuropathology occurs in areas of higher cognitive function and is thought to spread from the entorhinal cortex, basal forebrain, and nucleus basalis to the hippocampus and neocortex during the course of the disease [21,22]. Extensive neurodegeneration in these areas ultimately leads to a pronounced loss of brain volume in AD patients [2,18]. A striking feature of neurons with NFTs and neurons in the vicinity of amyloid plaques is a destruction of synapses, in turn resulting in a marked decrease in levels of neurotransmitters, including acetylcholine, serotonin, norepinephrine, and dopamine. In addition, dysregulation of the neurotransmitter glutamate contributes to synaptic deterioration and neuronal cell death via mechanisms related to excitotoxicity.

A subset of drugs currently used to treat AD patients is cholinesterase inhibitors. These agents, including donepezil, rivastigmine, and galantamine, block the hydrolysis of acetylcholine catalyzed by the enzyme acetylcholinesterase, thereby compensating for the loss of acetylcholine that results from the degeneration of cholinergic nerve terminals in AD [23]. Galantamine is also thought to enhance the action of acetylcholine on nicotinic receptors by acting as an allosteric modulator [24]. Another drug used to treat AD, memantine, is an uncompetitive inhibitor of *N*-methyl-D-aspartate receptors that acts by down-regulating glutamate-mediated neurotransmission [25]. Although glutamate is an excitatory neurotransmitter that plays an essential role in learning and memory, excess glutamate signaling leads to excitotoxicity, a mechanism that can result in neuronal death. Unfortunately, memantine and the cholinesterase inhibitors provide only a modest, temporary relief of AD symptoms, and they have no impact on the relentless progression of the disease [3].

B Genetics and AD

The fact that aggregates formed by Aβ peptides and tau are both observed in postmortem AD brains led to a prolonged debate in the field regarding which of these two polypeptides plays a more significant role in pathogenesis. Several genetic findings support the idea that Aβ plays a central role in neurodegeneration in the brain of AD patients [2,18]. First, mutations in the gene encoding APP are linked to early onset AD, and these mutations promote the cleavage of Aβ from APP or favor the production of the more fibrillogenic $A\beta_{42}$ peptide relative to the shorter, less aggregation-prone $A\beta_{40}$ variant. Second, trisomy 21 (Down's syndrome) is associated

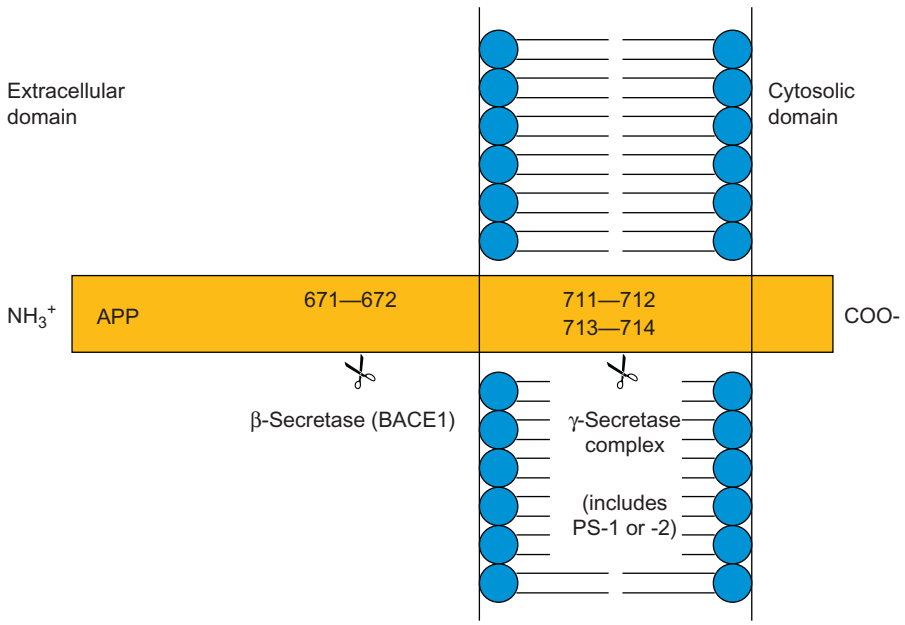

FIGURE 18.1 Diagram illustrating how Aβ peptides are released from APP via successive β- and γ-secretase cleavages. APP is shown as a transmembrane protein in the phospholipid bilayer (phospholipid headgroups are shown as filled circles; acyl chains are depicted as "tails" associated with each headgroup). The cleavage sites are shown in the representation of APP: β-secretase cleaves between residues 671 and 672, and γ-secretase cleaves between residues 711 and 712, yielding $A\beta_{1-40}$, or 713−714, yielding $A\beta_{1-42}$. PS, presenilin. *Adapted from J.-C. Rochet, Novel therapeutic strategies for the treatment of protein-misfolding diseases, Expert Rev. Mol. Med. 9 (2007) 1−34.*

with an AD-like phenotype in the fourth decade of life, and the APP gene is located on chromosome 21, further suggesting that an increase in Aβ levels can lead to AD-related neurodegeneration. Third, mutations in the gene encoding presenilin-1 or -2, proteins that constitute part of the γ-secretase complex involved in cleaving Aβ from APP (Fig. 18.1), are linked to early onset AD. Because presenilin mutations lead to an increase in levels of $A\beta_{42}$ relative to $A\beta_{40}$ [18,26,27], this observation further supports the idea that Aβ plays a major role in AD pathobiology.

Mutations in the gene encoding tau have been linked to frontotemporal dementia (FTD), but not AD [2]. Nevertheless, tau dysfunction is also thought to contribute significantly to neurodegeneration in AD, perhaps as a toxic phenomenon that occurs downstream of Aβ aggregation. Tau hyperphosphorylation results in dissociation of the protein from microtubules, tau aggregation and NFT formation, and a disruption of the microtubular network that is essential for axonal transport and proper synaptic function [28]. Evidence suggests that Aβ-induced oxidative stress [29,30] or the binding of secreted Aβ oligomers to neuronal receptors [31] results in the activation of downstream intracellular kinases involved in hyperphosphorylating tau, including GSK3β, CDK5, Fyn, and FAK1. Other findings suggest that oligomers formed by $A\beta_{42}$ can bind directly to GSK3α, resulting in stimulation of its tau hyperphosphorylation activity [32].

AD risk is modulated by allelic variation in the gene encoding apolipoprotein E (ApoE), a protein responsible for transporting cholesterol in the form of low-density lipoprotein [2,18,33]. There are three ApoE isoforms as a result of polymorphisms in the ApoE gene: ApoE2, ApoE3, and ApoE4. Individuals with one or two *ApoE* ε4 allele encoding ApoE4 have an increased risk of AD, whereas inheritance of the *ApoE* ε2 allele encoding ApoE2 decreases AD risk. A number of mechanisms could account for the increase in AD risk associated with the ApoE4 allele, including (i) decreased Aβ elimination via ApoE-mediated clearance pathways, including cellular uptake of Aβ through ApoE receptors; (ii) increased Aβ aggregation mediated by ApoE−Aβ interactions; and (iii) increased production of neurotoxic ApoE proteolytic fragments that promote tau pathology and interfere with mitochondrial function [2,34].

C Mitochondrial Dysfunction and Oxidative Stress in AD

The postmortem brains of AD patients are characterized by mitochondrial functional deficits, including a decrease in the activity of cytochrome C oxidase (complex IV) as a result of mitochondrial DNA deletions [30,35−37]. A deficit in complex IV activity has also been observed in platelets from AD patients [38]. Additional findings indicate that levels of the peroxisome proliferator activated receptor-γ co-activator 1α (PGC-1α), a transcriptional co-activator involved in regulating mitochondrial biogenesis and cellular energy homeostasis [39−41], are reduced in AD brain [42,43]. Postmortem brains of AD patients are characterized by elevated protein oxidation, protein nitration, and lipid peroxidation in regions enriched with amyloid plaques and NFTs [37,44]. Proteomic analyses have led to the identification of a large number of oxidized proteins in AD brain [45,46]. The loss of ATP and the accumulation of reactive oxygen species (ROS) resulting from mitochondrial impairment are early events in AD pathogenesis that are likely to play a key role in synaptic degeneration in the brains of AD patients [19,47].

Transgenic mice expressing AD-linked mutant forms of APP exhibit a range of mitochondrial deficits, including abnormal mitochondrial morphology and decreased mitochondrial membrane potential, ATP levels, and complex IV activity [37,47,48]. These impairments are accompanied by evidence of oxidative stress, including the presence of the lipid peroxidation product 4-hydroxynonenal [49]. Additional evidence from studies in APP transgenic mice suggests that oligomeric Aβ accumulates in mitochondria [50], where it interacts with a number of targets including the mitochondrial matrix enzyme Aβ-binding alcohol dehydrogenase [51,52] and the mitochondrial permeability transition pore subunit cyclophilin D [53,54], thereby triggering a loss of mitochondrial membrane potential, defects in mitochondrial electron transport, and a buildup of ROS [30]. Data from experiments in a cell-free system suggest that Aβ can be imported into isolated mitochondria via the action of the translocase of the outer membrane (TOM) machinery [55]. Transgenic mice expressing a mutant form of tau (P301L) linked to FTD also show evidence of mitochondrial dysfunction, including a decrease in complex I activity and ATP levels, accompanied by an increase in oxidative stress [37,47,56]. Data obtained from studies of triple transgenic mice expressing mutant APP, tau, and presenilin suggest that Aβ and tau act in a concerted manner to cause mitochondrial functional deficits (via inhibitory effects on complex IV and complex I, respectively), leading to a disruption of oxidative phosphorylation and an increase in oxidative stress [47,57,58].

In addition to triggering oxidative stress by causing an impairment of mitochondrial function, Aβ can directly trigger a buildup of ROS in neurons. In one study, membrane-associated Aβ was shown to trigger oxidative stress, potentially via a mechanism involving Cu^{2+} binding to Aβ, lipid peroxidation, and the diffusion of ROS by-products of lipid peroxidation into the cytosol [46].

Additional findings suggest that Aβ elicits oxidative stress by activating NADPH oxidase, an enzyme that produces ROS [59].

D Glial Activation in AD

The activation of glial cells (microglia and astrocytes) plays an important role in the pathogenesis of AD. Under normal conditions, not only do microglia and astrocytes provide nutrients to neurons, but they also fulfill an immune surveillance role aimed at eliminating pathogens from the brain [60]. In response to various inflammatory stimuli, glial cells become activated and release pro-inflammatory cytokines (e.g., prostaglandins such as PGE_2, interleukins such as IL-1β, tumor necrosis factor-α (TNF-α)) that cause neuroinflammation (Fig. 18.2) [61]. Activated glial cells also release ROS (superoxide, H_2O_2) and reactive nitrogen species (RNS) (e.g., nitric oxide (NO), which combines with superoxide to form peroxynitrite) due to the activation of enzymes such as NADPH oxidase and nitric oxide synthase (NOS) [60,61]. The production and release of cytokines, ROS, and RNS is mediated in part by the transcription factor NF-κB. Although the activation of glial cells is a part of the brain's innate immune response, in cases of AD, PD (see below), and other neurodegenerative diseases, excessive glial cell activation can be deleterious to neurons (Fig. 18.2).

Evidence from postmortem studies indicates that astrocytes and microglia are located near amyloid plaques in the brains of AD patients and have morphologies consistent with an over-activated phenotype associated with chronic neuroinflammation [18,61–63]. Additional evidence of microglial activation has been obtained from positron emission tomography (PET) imaging studies of living AD patients [64]. These observations have led to the suggestion that Aβ induces neurotoxicity indirectly by activating microglia recruited to amyloid plaques, potentially as an early event in AD pathogenesis, in addition to causing damage to neurons directly [61]. Consistent with this idea, evidence of microglial/astrocytic activation and elevated cytokine expression has been obtained in APP-expressing transgenic mice and in mice receiving intracerebral injections of oligomeric Aβ [63,65,66]. Although glial activation may be part of a physiological defense mechanism aimed at the clearance of Aβ aggregates (e.g., perhaps via phagocytosis) [65,67], this process is evidently ineffective in AD brain. Instead, cytokines, ROS, and RNS released by over-activated glia lead to enhanced Aβ deposition and neurotoxicity, thus contributing to a vicious cycle of amyloid formation, glial over-activation, and neurodegeneration in the brains of patients [61,65,66].

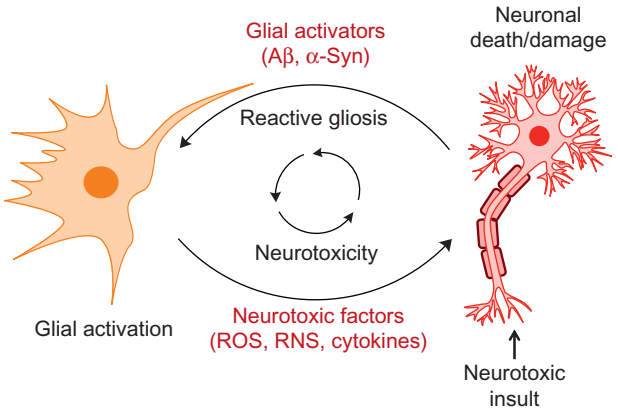

FIGURE 18.2 Model illustrating mechanisms of glial activation and neurotoxicity. Neurotoxic insults result in neuronal death or damage. Factors released from dying or damaged neurons (e.g., protein aggregates) can activate glial cells and result in the production of pro-inflammatory molecules including ROS, RNS, and cytokines. These agents can in turn damage neighboring neurons. Thus, a vicious cycle occurs between damaged neurons and activated microglia, resulting in progressive neurotoxicity. *Adapted from M.L. Block, L. Zecca, J.S. Hong, Microglia-mediated neurotoxicity: uncovering the molecular mechanisms, Nat. Rev. Neurosci. 8 (1) (2007) 57–69.*

III PARKINSON'S DISEASE

A Symptoms, Neuropathology, and Treatments

Characteristic motor symptoms of PD include the inability to initiate movement, resting tremor, rigidity of the limbs, and postural instability [68]. These symptoms result in large part from a loss of dopaminergic neurons from the *substantia nigra* pars compacta (SNpc) in the midbrain. Another neuropathological hallmark of PD is the presence of Lewy body inclusions enriched with fibrillar forms of the presynaptic protein α-synuclein (aSyn). Molecular events that are thought to play a role in neurodegeneration in PD include oxidative stress [69,70], loss of mitochondrial function [71–73], aSyn aggregation [74–76], and neuroinflammation [61].

Current PD therapies only temporarily alleviate symptoms and do not reverse the underlying neurodegeneration [77]. Dopamine replacement therapies (DRTs) are currently the most common therapeutic options available to PD patients. DRT medications include L-DOPA or synthetic dopamine receptor agonists, both of which are designed to rescue deficits in striatal dopamine neurotransmission. Patients on long-term DRT eventually experience highly disruptive side effects that include L-DOPA-induced dyskinesia, aggression, and/or insomnia [78]. In addition, multiple studies and case reports suggest that there is a risk of dependence and withdrawal in patients abusing their DRT medication [78–80].

Patients and health care providers are in need of safe and effective neuroprotective therapies that can stop or slow the neurodegenerative process.

The seeds of *Mucuna pruriens* (velvet beans) contain high levels of L-DOPA and are used as a PD therapy. Interestingly, clinical studies suggest that this plant may be equally or more effective than the standard L-DOPA therapy [81–83]. A number of studies suggest that a *M. pruriens* extract can not only provide a significant relief of tremor symptoms as a result of its L-DOPA content, but it can also slow the neurodegenerative process due to its neuroprotective activities [84,85]. For example, a *M. pruriens* extract was found to alleviate parkinsonian symptoms to a greater degree than a standard L-DOPA therapy in 6-OHDA-lesioned rats by both restoring motor function and protecting mitochondrial complex I activity [84]. Moreover, an extract prepared from *M. pruriens* seeds was found to alleviate plasmid DNA and genomic DNA damage induced after incubation with L-DOPA [86].

B Genetics and PD

It is estimated that familial cases of PD only represent 10% of the patients, whereas the majority of the cases are sporadic. Mutations in more than 20 genetic loci leading to an increased risk of developing the disease (referred to as "PARK" loci) have been identified [87–89]. The affected genes are involved in critical cellular processes such as regulating mitochondrial function and mitophagy (PARK2 (parkin) and PARK6 (PINK1)) [90–92], oxidative stress (PARK7 (DJ-1)) [93,94], or neurite outgrowth (PARK8 (LRRK2)) [95–98]. In addition, several mutations in the SNCA gene encoding the presynaptic protein aSyn, including substitutions encoding the variants A30P, E46K, H50Q, G51D, A53E, and A53T [5,99–105] and multiplications [106,107], are linked to familial cases of PD. Evidence suggests that mutant forms of aSyn have a higher propensity to undergo membrane-induced aggregation [108] or accelerated fibrillization in the absence of membranes (Fig. 18.3) [109]. Despite evidence of a role for genetics in determining PD risk, the development of the disease seems to be multifactorial, involving additional factors such as environmental exposure to toxicants and the influence of diet [110].

C Environmental Risk Factors and PD

Epidemiological evidence suggests that exposure to the environmental toxins rotenone and paraquat (PQ) is linked to an increased risk of developing PD [111–116]. Importantly, both of these agents are used extensively to model aspects of PD-related neurodegeneration in cellular and animal models of the disease (see later). Rotenone, a

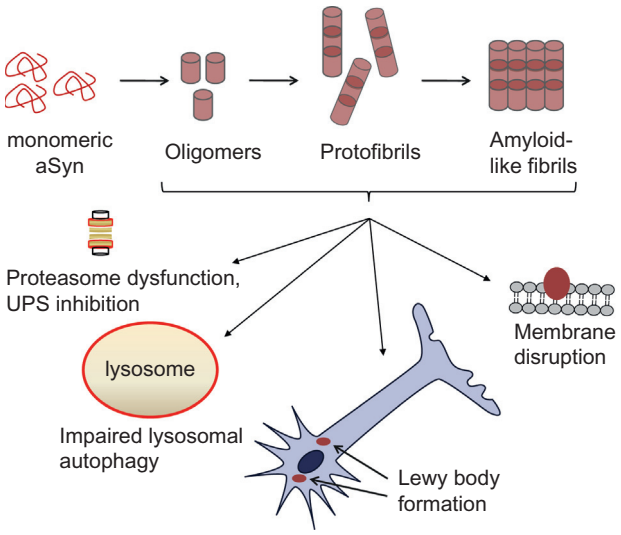

FIGURE 18.3 Model illustrating mechanisms of aSyn aggregation and neurotoxicity. aSyn is a natively unfolded protein that undergoes self-assembly under pathological conditions. aSyn aggregation involves the formation of intermediate species named protofibrils that undergo further assembly into larger amyloid-like fibrils. These fibrillar species are the main constituents of pathological inclusions named Lewy bodies. aSyn aggregation leads to membrane disruption, impairment of lysosomal autophagy, and UPS inhibition.

naturally occurring isoflavonoid extracted from plant material, is used as a pesticide [117,118]. Rotenone is a mitochondrial toxin that diffuses through the cell membrane to target the proton-translocating NADH:quinone oxidoreductase enzyme of the mitochondrial electron transport chain, referred to as complex I. Complex I is a large transmembrane protein complex composed of 45 subunits that is located in the mitochondrial inner membrane [119]. It is responsible for the transfer of electrons from NADH to ubiquinone, the first step in the series of reduction-oxidation (redox) reactions involved in mitochondrial respiration and ATP production [120–122]. Complex I is composed of two sites for quinone reduction, a hydrophobic site and a hydrophilic site, and it is the former that is targeted by rotenone. Under normal conditions, proton translocation at complex I occurs via a redox reaction that results from quinone reduction at the hydrophobic site. However, rotenone inhibits the NADH: quinone oxidoreductase activity at this hydrophobic, energy-transducing site of complex I [123,124]. The hydrophilic site of quinone reduction is nonenergy transducing and is not targeted by rotenone. Disruption of electron flux by rotenone leads to oxidative damage via the generation of superoxide (Fig. 18.4) [125–128]. In addition, rotenone-mediated complex I inhibition results in disruption of the cellular bioenergetic status as a consequence of impaired oxidative phosphorylation and ATP production [127]. Exposure to rotenone also leads to a

collapse of the mitochondrial membrane potential and a release of cytochrome C, a key initiating event of cellular apoptosis [129,130]. Rotenone treatment of cells expressing a rotenone-insensitive, single-subunit NADH dehydrogenase does not result in mitochondrial dysfunction, oxidative damage, or cell death, highlighting the essential role of complex I as the key target involved in rotenone toxicity [127].

Rotenone is used as a toxicant in a number of preclinical models that reproduce critical features of PD. Chronic systemic injection of rotenone in rats results in nigrostriatal damage with severe striatal denervation and a selective loss of dopaminergic neurons, particularly in the SNpc [73,131,132]. Rotenone-infused rats undergo selective targeting of complex I in all brain regions, leaving complex II and complex IV unaffected. In addition, the formation of aSyn-containing cytoplasmic inclusions in nigral neurons of rotenone-treated rats reproduces a hallmark feature of PD. Rats chronically treated with rotenone also develop behavioral deficits relevant to motor symptoms of PD such as tremor, rigidity, and reduced balance [73,132,133]. The exposure of neuroblastoma cells to rotenone results in impairment of mitochondrial respiration, ATP depletion, oxidative stress, and dose-dependent cell death [127,130,134]. However, ATP depletion does not seem to be a driving event leading to cell death, based on the observation that treating neuroblastoma cells with 2-deoxy-D-glucose depletes cellular ATP without generating oxidative stress but does not result in cell death [127]. Additional evidence from studies in cell culture suggests that oxidative phosphorylation inhibitors that do not affect complex I but deplete ATP without generating oxidative stress are nontoxic [134]. The fact that oxidative damage plays a critical role in rotenone neurotoxicity and the PD-like phenotype in cellular and animal models of rotenone exposure supports the rationale for discovering antioxidant therapies to stop or slow the neurodegenerative process [125,127].

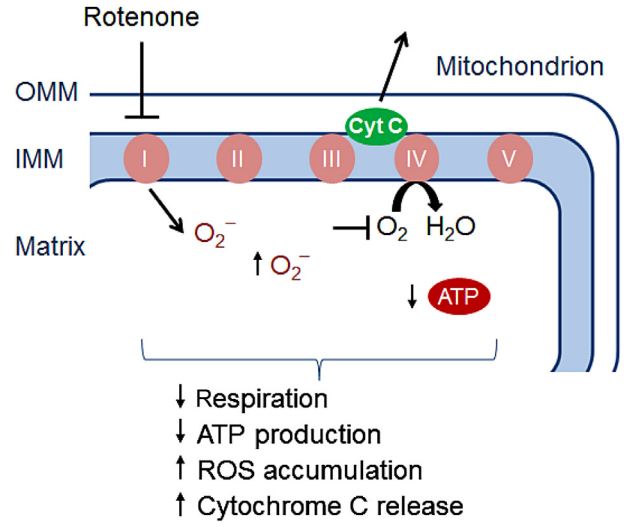

FIGURE 18.4 Model illustrating the mechanism of rotenone-mediated oxidative stress. Rotenone diffuses freely through the cell membrane and outer mitochondrial membrane (*OMM*) to the inner mitochondrial membrane (*IMM*), where it inhibits complex I of the electron transport chain. A decrease in complex I activity leads to electron leakage, a buildup of *ROS* (e.g., superoxide, O_2^-), disruption of oxygen consumption, depletion of *ATP*, and the release of cytochrome C into the cytosol.

The bipyridyl herbicide PQ (1′-dimethyl-4, 4′-bipyridinium dichloride) is a pro-oxidant that belongs to a family of redox-reactive heterocycles. PQ is converted from its native dicationic form (PQ^{2+}) to its monocationic form (PQ^+) by engaging in redox cycling reactions with molecular oxygen (Fig. 18.5) [135–137]. These redox cycling reactions, catalyzed by the cellular oxidases NADPH oxidase and NOS, lead to the generation of toxic free radicals and superoxide, initiating cellular oxidative stress and a cascade of events culminating in oxidative damage to DNA, protein, and lipids and eventually cell death [138,139]. Because PQ is structurally similar to the dopamine transporter (DAT) substrate MPP^+ (a toxin with selective toxicity to dopaminergic neurons—see later), it has been hypothesized that DAT plays a key role in shuttling PQ in dopaminergic neurons [140]. Additional evidence suggests that the organic cation transporter-3 (ORT-3) is involved in PQ toxicity [140]. ORT-3, a bidirectional transporter expressed in nondopaminergic cells such as astrocytes and GABAergic neurons, is thought to be involved in modulating the local concentration of toxic cationic species such as PQ near the cell body and synaptic terminals of midbrain dopaminergic neurons [141].

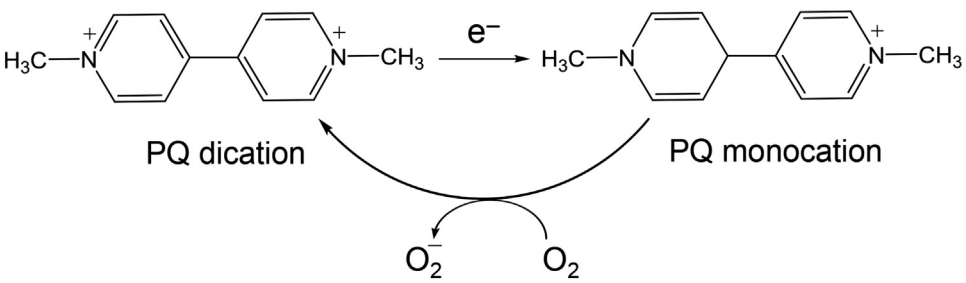

FIGURE 18.5 Schematic illustrating the mechanism of PQ-mediated oxidative stress. A redox cycling reaction involving the interconversion of monocationic and dicationic forms of PQ results in the production of ROS.

A number of studies have demonstrated that PQ triggers a PD-like phenotype in rodents. Male C57BL/6 mice exposed to a combination of PQ and the fungicide Maneb were found to undergo enhanced nigrostriatal dopaminergic neurotoxicity compared to animals exposed to either compound alone [142–144], a significant finding that yields insight into the role of multipesticide exposure in modulating PD risk. Mice treated with intraperitoneal PQ injections were characterized by PD-associated motor symptoms including reduced balance [145], a selective loss of nigrostriatal dopaminergic neurons [138,144], and evidence of aSyn aggregation [146]. Although cellular mechanisms contributing to PQ-mediated neurodegeneration are not fully understood, the pro-oxidant activity of PQ is thought to play a major role in nigrostriatal damage. In mice systemically injected with PQ, the loss of dopaminergic neurons was associated with an increase in lipid peroxidation and tyrosine nitration elicited by RNS [147].

D Mitochondrial Dysfunction and Oxidative Stress in PD

Mitochondria are central organelles that maintain the bioenergetic status of cells. Several observations suggest that mitochondrial dysfunction plays a key role in PD pathogenesis. First, the postmortem brains of PD patients are characterized by a decrease in complex I activity [148]. Second, human exposure to mitochondrial toxins is associated with an increase in PD risk, and animals treated with toxins that disrupt mitochondrial function develop a PD-like phenotype [116,127,149,150]. Third, mutations in a number of genes linked to familial PD are thought to cause mitochondrial impairment [92,151]. Mitochondrial functional deficits arising from environmental exposures or gene mutations include inefficient electron transport (resulting from a leakage of electrons from the transport chain) and a collapse of mitochondrial membrane potential, leading to ROS accumulation, oxidative damage, depletion of cellular ATP, a release of cytochrome C, and ultimately neuronal cell death [73].

Multiple observations provide pathological evidence for mitochondrial dysfunction in PD. Analysis of the mitochondrial electron transport chain in postmortem brains of PD patients revealed a selective loss of activity of complex I (but not in other complexes) in the SNpc and prefrontal cortex [148,152]. Defects in mitochondrial function and complex I activity have been reported in other tissues of PD patients including fibroblasts [153], platelets [154], and skeletal muscle [155]. Moreover, the brains and peripheral tissues of PD patients are characterized by extensive oxidative damage to macromolecules including proteins, lipids, and nucleic acids [70,73].

Toxins that impair mitochondrial function are thought to increase the risk of PD in humans and have been used to develop animal models of PD. As outlined earlier, rotenone is epidemiologically associated with an increase in PD risk in humans, and it triggers a PD-like phenotype that reproduces PD-like features in rats [73,116]. A study of individuals who developed severe parkinsonism after self-administering preparations of synthetic meperidine analogs contaminated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) led to the discovery of MPTP as a PD-related neurotoxin [156–158]. MPTP crosses the blood brain barrier (BBB) and is metabolized to its active form MPP^+ by monoamine oxidase B in astrocytes. MPP^+ is then shuttled from the extracellular space into dopaminergic neurons by DAT [159]. Evidence suggests that MPP^+ mediates its toxicity largely by targeting complex I in the mitochondrial electron transport chain [160–162].

An extensive body of evidence supports the idea that the accumulation of wild-type or mutant aSyn can lead to mitochondrial dysfunction. The brains of transgenic mice expressing human A53T aSyn, which develop a motor phenotype characteristic of PD, are characterized by mitochondrial damage and degeneration [163]. Transgenic mice over-expressing wild-type aSyn show evidence of enhanced mitochondrial degeneration in the SNpc compared to nontransgenic mice after treatment with MPTP [164]. A number of groups have reported that aSyn is localized to mitochondria when over-expressed in neuronal cell lines [165,166]. Depletion of mitochondrial DNA abrogates aSyn toxicity in aging yeast, suggesting that functional mitochondria are involved in mediating the toxic effects of aSyn in this model [167]. It has also been reported that an anomalous, high-affinity interaction between posttranslationally modified forms of aSyn and the outer membrane translocase protein TOM20 results in deficient mitochondrial protein import, impaired electron transport, enhanced ROS accumulation, and a decrease in mitochondrial membrane potential [168].

Loss-of-function mutations in the gene encoding DJ-1, a molecular chaperone and oxidative stress sensor [169–171], are associated with early-onset PD [93]. In addition, wild-type DJ-1 is oxidized to a greater extent in the brains of patients with sporadic PD than in age-matched controls [172]. A loss of DJ-1 function results in increased oxidative stress or sensitivity to oxidative insults in cellular and animal models [171,173–175]. Multiple lines of evidence suggest that DJ-1 is involved in modulating mitochondrial function. DJ-1 can transfer from the cytosol to mitochondria in cells subjected to oxidative stress, and this mitochondrial localization is regulated by the oxidation status of cysteine residue C106 [176]. *Drosophila* that lack functional DJ-1 are more sensitive to oxidative stress and the mitochondrial toxin

rotenone [171]. Brains of DJ-1 knockout mice show evidence of fragmented mitochondria, resulting in increased sensitivity to oxidative stress and neuronal cell death [177]. Additional findings suggest that deletion of the DJ-1 gene results in increased mitochondrial H_2O_2 production [178], and DJ-1 up-regulation has been shown to rescue a loss of function of PINK1, a protein involved in regulating mitophagy [179].

E Vulnerability of Nigral Dopaminergic Neurons in PD

Considerable effort in PD research has been focused on understanding why dopaminergic neurons of the SNpc undergo selective degeneration in the brains of patients. One explanation appears to be that dopaminergic neurons have relatively high basal levels of ROS, including hydrogen peroxide (H_2O_2) and hydroxyl radicals (˙OH), resulting from the metabolism and auto-oxidation of cytosolic dopamine [73,180,181]. In turn, these ROS can cause damage to cellular macromolecules, ultimately leading to cell death. Oxidatively modified forms of aSyn [182–186] and adducts formed by reaction of the protein with oxidized derivatives of dopamine [187–191] have been reported to have a high propensity to form potentially toxic aggregates. Dopaminergic neurons in the SNpc are more vulnerable than other dopamine-producing neurons in nearby brain structures, in part because nigral neurons have lower levels of the vesicular monoamine transporter-2, the transport protein responsible for dopamine uptake from the cytosol into vesicles [192]. In addition, dopaminergic neurons in the SNpc selectively express the L-type Ca(v)1.3 channel, and calcium entry through this channel results in a build-up of intracellular Ca^{2+} that in turn imposes a metabolic burden on nigral dopaminergic neurons, resulting in mitochondrial dysfunction and oxidative stress [175,193–195]. As a result of these multiple factors, nigral dopaminergic neurons are thought to have high basal levels of ROS that render them more vulnerable to further oxidative insults compared to other neuronal populations subjected to lower basal levels of oxidative stress.

F Glial Activation in PD

Glial activation plays an important role in PD pathogenesis [61,196–198]. Postmortem PD brains show evidence of microglial activation and increased levels of proinflammatory molecules [199], and activated microglia have been detected in the brains of living PD patients via PET imaging [200]. PD-related toxins such as rotenone [201,202] and PQ [203], as well as aSyn-encoding virus [204] and exogenous aSyn aggregates [205], have been shown to cause glial activation in cellular and animal models. ROS and aSyn oligomers produced and released by dopaminergic neurons are thought to trigger microglial/astrocytic activation, resulting in the release of proinflammatory molecules, ROS, and RNS from these cells and leading to a vicious cycle of neurotoxicity and glial activation [61,205–208]. Dopaminergic neurons may be particularly vulnerable to glial activation given the abundance of microglial cells in the SNpc [209]. Interestingly, lipopolysaccharide (LPS), a classic inflammatory agent that triggers astrocyte and microglial activation, has been shown to induce dopaminergic cell death [210,211], providing further evidence of a role for glial activation in the loss of dopaminergic neurons.

Additional observations suggest that nigral susceptibility to neuroinflammation is increased in animals exposed to peripheral inflammatory insults, including dextran sulfate sodium, an agent that triggers ulcerative colitis [212]. Another potential connection between nigral degeneration and peripheral inflammation is implied by the fact that LRRK2 is genetically linked to both PD and inflammatory bowel disease [213,214]. Collectively, these observations suggest that interactions between systemic inflammation and inflammation in the brain contribute to neurodegeneration in PD.

IV DIETARY PLANTS AND NEURODEGENERATIVE DISEASE

A Health-Promoting Plant Constituents

A number of phytochemicals are thought to play a major role in the health-promoting activities of herbal medicines. As one example, broccoli and other cruciferous vegetables are of great interest due to their unique chemical composition. Extensive research findings suggest that cruciferous vegetables and their main constituents, including glucosinolates and their hydrolysis products, isothiocyanates, have remarkable health benefits. Each type of cruciferous vegetable contains different glucosinolates that are hydrolyzed to different isothiocyanates by the bacterial microflora of the gastrointestinal tract [215]. Among the most studied of the isothiocyanates, sulforaphane (derived from glucoraphanin, found in broccoli) and allyl isothiocyanates (derived from sinigrin, also found in broccoli) show exceptional health-promoting qualities in various disease models [216–218].

Garlic belongs to the botanical family of Amaryllidaceae, along with onions, chives, and garlic chives. Garlic is characterized by high contents of various garlic organosulfur compounds including sulfur compounds derived from alliin (L-(+)-S-allylcysteine sulfoxide) such as allyl thiosulfinates, and sulfur compounds not derived from alliin such as γ-glutamyl-*S*-allylcysteine [219]. Garlic organosulfur compounds are responsible for many health benefits in multiple

disease states such as neurodegenerative diseases, cancer, and diabetes [215,220].

Polyphenols are the largest group of secondary plant metabolites with more than 8000 phenolic structures identified [221]. Polyphenols have chemical structures characterized by the presence of multiple phenolic groups and exist as aglycones or (more typically) as glycosidic derivatives. Polyphenols in the aglycone form are classified into two main classes, flavonoids and nonflavonoids, based on the number and arrangement of their aromatic rings (Fig. 18.6). The chemical skeleton of flavonoids consists of two benzene rings (referred to as ring A and ring B) connected with a carbon bridge forming a pyran ring (referred to as ring C) (Fig. 18.7) [222]. Flavonoids are further classified into subgroups including the flavonols (e.g., quercetin, isorhamnetin), isoflavones (e.g., genistein, daidzein), and anthocyanidins (e.g., cyanidin, delphinidin) on the basis of differences in the structure of ring C and the degree of hydroxylation of rings A and B (Fig. 18.6) [221]. The ubiquitous flavonol aglycone quercetin has been identified in a variety of fruits, vegetables, cocoa products, beverages, and cereals in 279 different glycosidic forms [221]. The flavonol isorhamnetin is found in its glycosylated and aglycone form in fruits, beverages, and nuts. Isoflavones are the main active principles of soy products and are found in medicinal plants such as red clover. Cultivated or wild berries such as blueberry, elderberry, or chokeberry are characterized by a dark red or black color resulting from large concentrations of anthocyanidin glycosides, termed anthocyanins (ANCs).

Nonflavonoid polyphenols are classified into the three subgroups: (i) phenolic acids, which consist of an aromatic ring with a carboxylic acid functional group; (ii) stilbenes, members of the phenylpropanoid family with a $C_6-C_2-C_6$ structure; and (iii) lignans, which have a characteristic dibutylbenzene skeleton (Figs. 18.6 and 18.7). Two important representatives of the phenolic acid and stilbene classes, curcumin and resveratrol (respectively), are discussed in more detail later.

B Epidemiology

The health-promoting effects of polyphenol-rich foods in neurodegenerative disorders have been validated by a number of epidemiological studies [9,10]. Some groups have reported that the consumption of wine or dietary flavonoids is associated with a reduced risk of AD or cognitive decline [11–14], and the consumption of polyphenol-rich juices may delay the onset of AD in carriers of the *ApoE* ε4 allele [15]. The results of one study suggest that the regular consumption of berries enriched with ANC or proanthocyanidins (PAC) is associated with a lower risk of developing PD [16]. Additional evidence suggests that the incidence of PD is reduced as a result of elevated tea consumption [17]. Collectively, these observations imply that the consumption of polyphenols can potentially decrease the risk of neurodegenerative disease and slow pathological decline of the aging brain.

C Brain Bioavailability

The BBB physically separates the circulating blood from the brain extracellular fluid and is formed by endothelial cells supported by various other types of cells including astrocytes [223]. The BBB enables the diffusion of factors that support brain development and function while preventing the entry of potentially harmful factors [224]. A number of polyphenols or polyphenol metabolites have

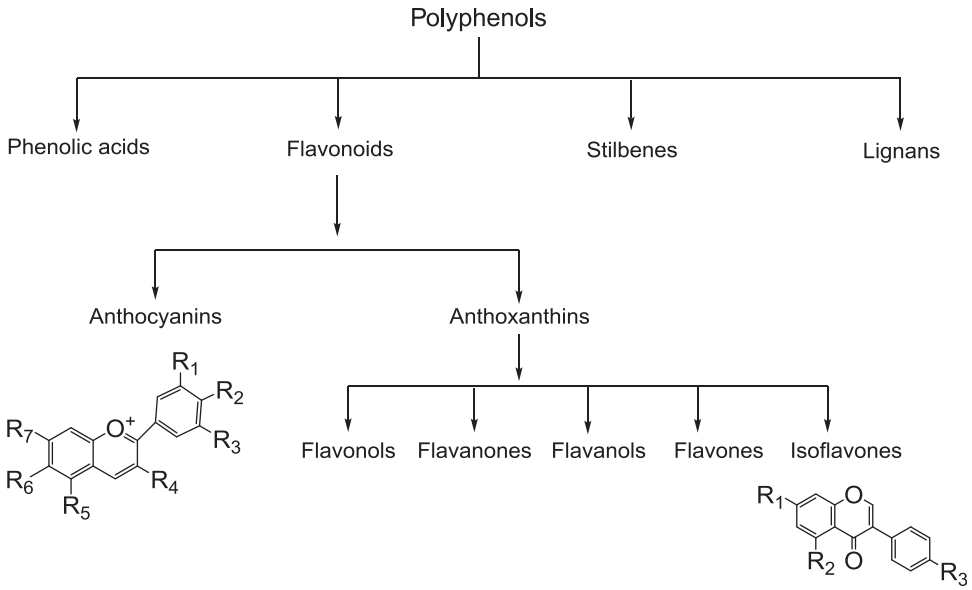

FIGURE 18.6 Classification of polyphenols, showing generic chemical structures of ANCs and isoflavones.

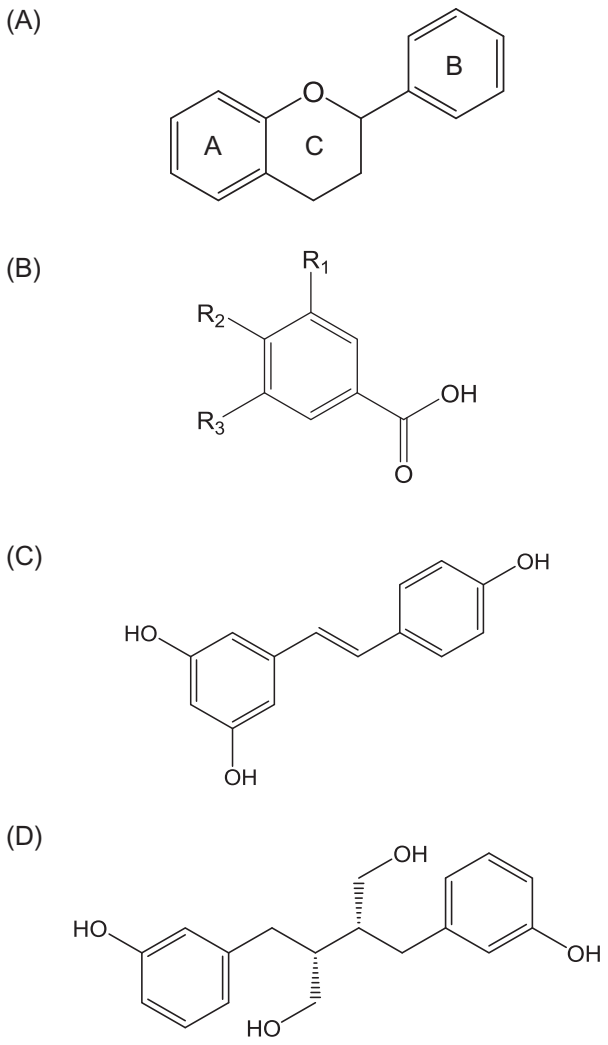

FIGURE 18.7 Chemical structures of different classes of polyphenols. The structures are of a generic flavonoid (A), showing the A, B, and C rings; a generic phenolic acid (B); the stilbene resveratrol (C); and the lignan enterodiol (D).

been found to penetrate the BBB in mammalian models [225]. Brain levels of total ANC from rodents fed a blackberry ANC-rich diet (14.8 mmol ANC per kg diet, 25 g diet/rat/day) were found to be 0.25 ± 0.05 nmol/g of brain tissue [226]. This concentration was lower than that in the plasma, liver, or kidney. In another study, significant amounts of ANC were detected in brain tissue from Yorkshire × Landrace male pigs fed a diet enriched with a blueberry extract (1%, 2%, and 4% (w/w)) [227]. One of the anthocyanidins examined in this study, malvidin, accounted for approximately 50% of the total ANC concentration in the cortex. Consistent with these results, rats fed a 2% (w/w) blueberry-supplemented diet were found to accumulate ANC in the brain, with malvidin derivatives exhibiting enhanced brain bioavailability compared to other ANCs [228]. A number of other polyphenolic constituents, including isoflavones (as either the intact forms or metabolites), quercetin, kaempferol, isorhamnetin, organosulfur compounds, and sulforaphane, have been shown to penetrate the BBB [219,229–235]. Collectively, these observations suggest that some polyphenols can cross the BBB and accumulate in brain tissue, albeit at relatively low levels. Extracts enriched in these polyphenols could potentially lower the risk of neurodegenerative disease or slow disease progression in patients. Because polyphenols accumulate in the brain at lower levels compared to endogenous antioxidants, their mechanisms of neuroprotection are likely mediated via the activation of cellular protective pathways rather than by radical scavenging [236].

V NEUROPROTECTIVE EFFECTS OF PHYTOCHEMICALS IN AD MODELS

A Anthocyanins

A number of ANC-rich extracts have been found to interfere with neuronal cell death in models of Aβ neurotoxicity relevant to AD. In one study, a blueberry extract was reported to alleviate neurotoxic effects of Aβ in a primary hippocampal neuron model [237]. Intragastric administration of black soybean ANC led to a decrease in mitochondrial apoptotic markers in the hippocampus of rats receiving intracerebroventricular injections of $A\beta_{42}$ [238]. An ANC-rich bilberry extract was shown to alleviate cognitive deficits in transgenic mice expressing APP and mutant presenilin-2, apparently via a mechanism involving a redistribution of Aβ into a nontoxic, aggregated form [239]. Another study revealed that extracts prepared from bilberries and blackcurrants attenuated spatial working memory deficits exhibited by transgenic APP/presenilin-1 mice, and this cognitive improvement correlated with a decrease in levels of soluble $A\beta_{40}$ and $A\beta_{42}$ or the ratio of insoluble $A\beta_{42}$ to $A\beta_{40}$ in the brains of bilberry- or blackcurrant-treated mice, respectively [240]. The ANC cyanidin-3-*O*-glucoside (C3G) was found to alleviate deficits in learning and memory in rats receiving intrahippocampal injections of aggregated Aβ, and this protective effect correlated with a decrease in GSK-3β activation and tau hyperphosphorylation in the brains of these animals [241].

B Proanthocyanidins

A PAC-rich grape seed extract consisting of catechin, epicatechin, and epicatechin gallate was found to alleviate deficits in cognitive ability and spatial memory exhibited by transgenic mice expressing mutant APP and PS1 [242]. Another study revealed that Tg2576 transgenic

mice expressing the Swedish mutant form of APP (KM670/671NL) have less pronounced cognitive deficits when dosed orally with monomeric but not polymeric PAC, and only monomeric PAC metabolites (glucuronide derivatives) can be detected in rodent brain [243,244].

C Stilbenes

Multiple lines of evidence suggest that resveratrol can alleviate neurodegeneration in rodent models of AD. In one study, resveratrol was found to alleviate spatial memory deficits and hippocampal neuronal damage in rats receiving intracerebral injections of aggregated forms of an Aβ fragment spanning residues 25−35 ($A\beta_{25-35}$) [245]. Additional research revealed that orally administered resveratrol interfered with Aβ accumulation, amyloid plaque burden, and microglial activation in the brains of transgenic mice expressing mutant APP with or without mutant presenilin-1 [246−248]. Another stilbene, tetrahydroxystilbene glucoside, was shown to attenuate deficits in learning memory in APP-expressing transgenic mice [249] and ameliorate memory impairment and synaptic degeneration in rats receiving intracerebral injections of $A\beta_{42}$ [250].

D Isoflavones

Soy isoflavones have been shown to ameliorate deficits in learning and memory in rats subjected to intracerebroventricular administration of $A\beta_{42}$ [251]. Another study revealed that the soy isoflavone genistein alleviates short-term spatial memory defects and hippocampal oxidative damage in rats receiving intracerebral injections of $A\beta_{40}$ [252]. Oral administration of puerarin, an isoflavone isolated from the root of *Pueraria*, led to an attenuation of cognitive impairment exhibited by transgenic mice expressing mutant APP and presenlin-1 and to a decrease in oxidative damage in the brains of these animals [253]. Puerarin was also shown to interfere with cognitive deficits and hippocampal neurodegeneration in rats receiving intracerebral injections of $A\beta_{42}$ [254].

E Curcumin

Curcumin, a major component of the Indian spice turmeric (*Curcuma longa*) [221], was found to interfere with cell death in primary rat hippocampal cultures transduced with adenovirus encoding $A\beta_{42}$ [255]. Other groups have reported that oral or intraperitoneal administration of curcumin results in a decrease in memory deficits, neurotoxicity, and glial activation in rats receiving intracerebroventricular infusions of $A\beta_{42}$ [256]. Additional studies have revealed that orally administered curcumin attenuates amyloid plaque burden, accumulation of insoluble Aβ, and neuroinflammation in Tg2576 mice [257,258], and amyloid plaque size and neuritic anomalies were found to be reduced in transgenic APP/presenilin-1 mice receiving curcumin via tail vein injections [259]. Although several human clinical trials of curcumin in AD have been carried out, so far none of these have provided evidence for a neuroprotective effect of the compound in AD patients [260]. These negative findings are potentially a reflection of suboptimal study design and also the fact that curcumin has weak bioavailability.

VI NEUROPROTECTIVE EFFECTS OF PHYTOCHEMICALS IN PD MODELS

A number of polyphenol-rich extracts and individual polyphenols have been characterized in terms of their neuroprotective activities in PD models. Typically, these models consist of neuronal cells, primary mesencephalic cultures, or rodents exposed to PD-related insults, including rotenone, PQ, MPP^+ (in the case of cultured cells), or MPTP (in the case of rodents). Another PD-related neurotoxin, 6-hydroxydopamine (6-OHDA), is used extensively to model dopaminergic neurodegeneration in cell culture and in vivo [159,261,262]. Because 6-OHDA is structurally similar to dopamine, it preferentially targets dopaminergic neurons as a result of DAT-mediated uptake; 6-OHDA elicits neurotoxicity via the generation of ROS that causes oxidative damage [159,263], and it has also been reported to elicit mitochondrial dysfunction via the inhibition of complex I [261,264].

A Anthocyanins

A number of ANC-rich extracts and individual ANCs have been shown to attenuate dopaminergic cell death in cellular and animal models of PD. An ANC-containing mulberry extract from *Morus alba* L. (Moraceae) alleviated nigral degeneration in mice exposed to MPTP and in primary midbrain cultures exposed to 6-OHDA or MPP^+ [265]. In addition, ANC-rich extracts prepared from blueberries and blackcurrants, but not purple basil, interfered with dopaminergic cell death in primary midbrain cultures exposed to rotenone [266].

The ANC molecule pelargonidin was reported to attenuate motor defects and nigral degeneration in rats exposed to 6-OHDA [267]. Moreover, malvidin-3-*O*-glucoside, cyanidin-3-*O*-sophoroside, and delphinidin-3-*O*-glucoside, as well as a hibiscus extract consisting of cyanidin-3-*O*-sambubioside and/or delphinidin-3-*O*-sambubioside, were found to interfere with rotenone neurotoxicity in primary midbrain cultures [266]. A comparison of active versus inactive ANC in this model led to the conclusion that the identity of the sugar molecule attached to the ANC core is an important factor in determining the level of

neuroprotective activity. Together with evidence that ANCs are detectable as intact glycosides in the brains of rats [226,228,268–270] and pigs [236] fed ANC-rich diets, these observations suggest that ANCs have the potential to attenuate neurotoxicity in the brains of PD patients.

B Proanthocyanidins

A PAC-rich cocoa extract was found to interfere with nigral degeneration and a loss of striatal dopamine in 6-OHDA-treated rats, whereas catechin-rich grape seed extracts failed to alleviate neurotoxicity in this model [271]. Monomeric PAC such as catechin and (-)-epigallocatechin gallate (EGCG) and extracts that contain these molecules but lack polymeric PAC (e.g., tea extracts) interfered with neurodegeneration in cellular and rodent models of PD [272–275]. A PAC-rich grape-seed extract was shown to alleviate rotenone-mediated dopaminergic cell death in primary midbrain cultures [266]. Together, these findings suggest that a number of PAC-rich extracts and monomeric PAC constituents can attenuate neurotoxicity elicited by PD-related insults and could potentially have neuroprotective activity in the brains of PD patients.

C Stilbenes

An oxyresveratrol-rich mulberry extract and the pure stilbene resveratrol were found to interfere with rotenone-mediated dopaminergic cell death in primary mesencephalic cultures [266,276], and resveratrol attenuated ER stress, caspase-3 activation, and oxidative stress in the brains of rotenone-treated rats [277]. Multiple groups have shown that resveratrol ameliorates behavioral deficits exhibited by MPTP-treated mice and alleviates dopaminergic cell death, striatal dopamine depletion, and oxidative stress in the brains of these animals [278–282]. Resveratrol has also been shown to relieve motor deficits and neuropathology in 6-OHDA-treated rats [262,283]. Another study revealed that resveratrol suppresses dopaminergic cell death and microglial activation in primary midbrain cultures exposed to LPS [284]. Together with evidence that resveratrol glucuronide is present in the brains of mice fed a grape polyphenol mixture [270], these observations suggest that stilbenes or their metabolites may slow neurodegeneration in the brains of PD patients.

D Isoflavones

Isoflavones from *Trifolium pratense* have been shown to suppress dopaminergic cell death triggered by LPS in primary midbrain cultures [285]. In addition, nigral degeneration and striatal dopamine depletion were alleviated in 6-OHDA-lesioned rats fed an isoflavone-rich red clover diet [271]. Genistein [286,287] and puerarin [288,289] were reported to attenuate nigral dopaminergic cell death and the loss of striatal dopamine and dopamine metabolites in the brains of rats exposed to 6-OHDA, and in some of these studies the isoflavone treatment led to an amelioration of motor deficits. Genistein and puerarin were also shown to interfere with nigral degeneration and striatal dopamine depletion in the brains of MPTP-treated mice and to improve the motor function of these animals [290,291]. Biochanin A, an *O*-methylated isoflavone from chickpea, was found to alleviate PD-like features of rats receiving an intranigral unilateral injection of LPS, including motor deficits, nigral degeneration, and microglial activation [292]. A number of isoflavones or their metabolites penetrate the BBB in rats [232,233], suggesting that isoflavone-rich extracts and individual isoflavones could potentially be of clinical benefit by reducing PD risk or slowing neurodegeneration in the brains of patients.

E Curcumin

Several groups have reported that curcumin protects immortalized neuronal cells against cytotoxicity resulting from intracellular aSyn over-expression [293] or from exposure to extracellular pre-formed aSyn oligomers [294]. Other studies have revealed that dietary administration of curcumin leads to an amelioration of behavioral deficits exhibited by aSyn-expressing transgenic mice [295] or flies [296] and attenuates oxidative stress and apoptosis in the fly model. Curcumin has also been shown to interfere with nigral dopaminergic cell death and striatal dopamine depletion in MPTP-treated mice [297,298] and in 6-OHDA-treated rats [299–301].

F Other Phytochemicals

The methionine analog *S*-methyl-L-cysteine, a substrate of the methionine sulfoxide reductase A (MsrA)-mediated antioxidant system, was found to mitigate aSyn-mediated toxicity in *Drosophila* [302]. *S*-allylcysteine alleviated motor dysfunction, lipid peroxidation, striatal dopamine depletion, and the production of superoxide in MPTP-treated mice [303]. In addition, *S*-allylcysteine and aged garlic extract ameliorated oxidative damage in cellular and animal models by scavenging free radicals and inducing activation of the antioxidant response [304]. 6-Methylsulfinylhexyl isothiocyanate (6-HITC) and sulforaphane attenuated PQ-induced toxicity in rat striatal cultures [305]. Sulforaphane was also found to interfere with neurodegeneration in a *Drosophila* loss-of-function parkin mutant [306] and to attenuate motor deficits and nigral dopaminergic cell death in mice exposed to 6-OHDA [307].

VII NEUROPROTECTIVE MECHANISMS OF PHYTOCHEMICALS

A Induction of the Nrf2-Mediated Antioxidant Response

Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of more than 200 cytoprotective genes involved in cellular antioxidant and anti-inflammatory responses, including glutamate cysteine ligase (GCL), heme oxygenase 1 (HO-1), and NAD(P)H dehydrogenase quinone-1 (NQO1) [308]. In the cytoplasm, Nrf2 is sequestered by Kelch-like ECH-associated protein 1 (Keap1) and targeted for degradation by the ubiquitin-proteasome pathway (Fig. 18.8) [309]. Under conditions of oxidative stress, the interaction between Nrf2 and Keap1 is disrupted, resulting in Nrf2 stabilization and translocation to the nucleus where it binds the antioxidant response element (ARE) in the 5′ flanking region of its target genes [308,310–313]. In the brain, Nrf2 activity is prominent in astrocytes, which play a key role in neuronal antioxidant responses by producing and releasing glutathione (GSH) metabolites that are imported and reassembled by neighboring neurons (Fig. 18.9) [314]. Multiple lines of evidence indicate that activation of the Nrf2/ARE pathway alleviates neuropathology in the APP/PS1 transgenic mouse model of AD [315–317] and in toxin-based and genetic models of PD [306,313,318–323]. Hence, this pathway is considered as an excellent target for the design of antioxidant therapies with the potential to stop or slow neuronal cell loss in PD and other neurodegenerative diseases [324–326].

ANCs are largely responsible for the health-promoting benefits associated with regular consumption of various berries and berry extracts [16,327]. Not only can these ANCs cross the BBB (see earlier), but they also have potent antioxidant, antiaging, and neuroprotective activities [328–330]. Evidence from several reports suggests that ANC-rich berry extracts and individual ANCs activate the Nrf2 antioxidant response as part of their protective mechanism in various disease models [328,329,331]. A C3G-enriched fraction from Chinese Bayberry was found to induce Nrf2 nuclear translocation and HO-1 up-regulation in INS-1 cells [328]. HUVEC cells treated with serum from healthy individuals fed an ANC-supplemented diet showed increased nuclear translocation of Nrf2 and up-regulated expression of the Nrf2-regulated proteins HO-1 and NQO1 [329]. Interestingly, not all ANCs have the same capacity to activate Nrf2 signaling. For example, kuromanin, delphinidin, malvidin, and cyanidin were found to have higher propensities to induce up-regulation of Nrf2-mediated transcription compared to peonidin, pelargonidin, and petunidin in rat liver cells [331], suggesting that berries with different ANC compositions and concentrations likely differ in terms of their relative abilities to induce the Nrf2 response.

Evidence from multiple reports indicates that sulforaphane and other isothiocyanates activate the Nrf2-mediated antioxidant response. In one study, a microarray analysis led to the identification of genes up-regulated by sulforaphane in the small intestine of wild-type ($Nrf2^{+/+}$) and knock out ($Nrf2^{-/-}$) mice [332]. The transcriptional profile showed that sulforaphane modulates the transcription of numerous Nrf2 target genes, including glutathione-*S*-transferase (GST) and NQO1. Additional results demonstrated that (i) sulforaphane up-regulated striatal and ventral midbrain HO-1 and NQO1 levels in $Nrf2^{+/+}$ but not $Nrf2^{-/-}$ animals, and (ii) sulforaphane alleviated

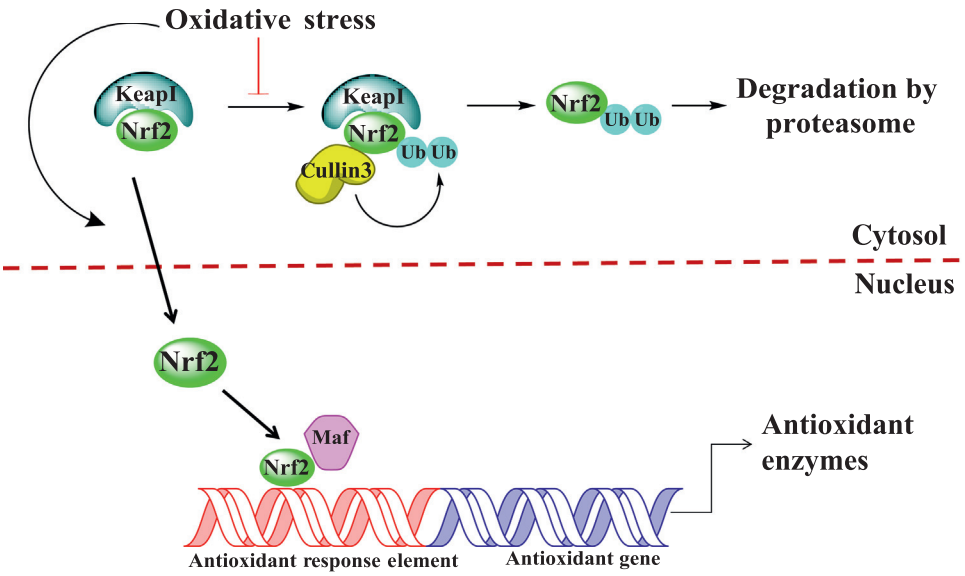

FIGURE 18.8 Schematic illustrating the classic mechanism of Nrf2 activation. Under normal conditions, Keap1 sequesters Nrf2 in the cytoplasm and regulates its ubiquitination by cullin-3 and subsequent degradation by the UPS. Under conditions of oxidative stress, the oxidation of cysteine residues on Keap1 results in its conformational change and the release of Nrf2. Nrf2 translocates to the nucleus, where it heterodimerizes with Maf, binds to the ARE, and activates the transcription of genes encoding antioxidant enzymes.

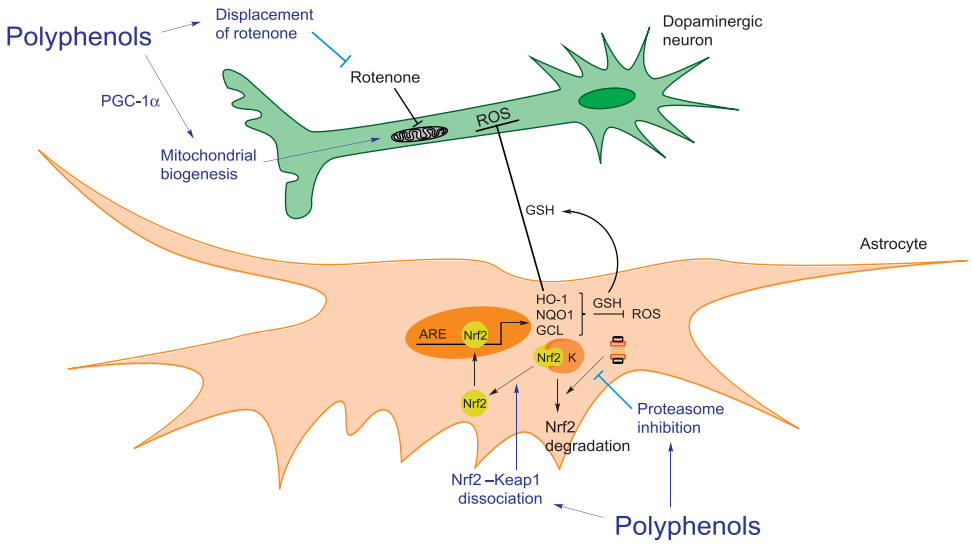

FIGURE 18.9 Model illustrating polyphenol-mediated antioxidant responses. Nrf2 levels increase in astrocytes as a result of ROS- or polyphenol-induced dissociation of Nrf2 from its repressor protein Keap1 ("K"), or as a result of polyphenol-mediated UPS inhibition. A build-up of astrocytic Nrf2 leads to an increase in the expression of genes involved in the cellular antioxidant response, including heme oxygenase 1 (HO-1), NQO1, and GCL, an enzyme involved in GSH synthesis. GSH subunits produced as a result of GSH metabolism are released from astrocytes and imported into neurons, where they are reassembled into GSH. Polyphenols can also induce an antioxidant response by ameliorating mitochondrial dysfunction (shown here in neurons exposed to rotenone) by (i) activating PGC-1α, a transcriptional co-activator that induces mitochondrial biogenesis and the expression of ROS-detoxifying enzymes; and (ii) displacing rotenone from complex I of the electron transport chain.

MPTP-induced dopaminergic cell loss in $Nrf2^{+/+}$ mice but not their $Nrf2^{-/-}$ littermates [235]. Sulforaphane was also shown to induce an increase in levels of GSH, a product of the Nrf2-mediated antioxidant response, in parkin loss-of-function flies [306] and in 6-OHDA-lesioned mice [307]. Another study revealed that 6-HITC and sulforaphane-induced Nrf2 nuclear translocation and up-regulation of GSH and HO-1 in rat striatal cultures exposed to PQ, although the increase in HO-1 levels was not necessary for the observed neuroprotective effects in these cultures [305]. Finally, mouse Hepa-1c1c7 cells and primary cortical neurons treated with a broccoli seed extract, sulforaphane, or allyl isothiocyanates exhibited an increase in Nrf2 antioxidant activity and the transcription of Nrf2 target genes encoding HO-1 and/or NQO1 [333,334].

Evidence suggests that extracts prepared from aged garlic (*Allium sativum*), as well as organosulfur compounds in these extracts, elicit antioxidant effects by activating the Nrf2 response. One organosulfur constituent of garlic extract, *S*-allyl-cysteine, was found to induce an increase in Nrf2 expression in rat cortex [304] and to alleviate neuronal damage in cellular and animal models of ischemia by inducing Nrf2-mediated transcription [335]. In addition, *S*-allyl-cysteine was reported to activate Nrf2-mediated transcription in rat striatum, an activity that could account for the ability of the compound to attenuate striatal neurotoxicity in the rat 6-OHDA model [336]. Garlic constituents have also been shown to activate the Nrf2/ARE pathway in nonneuronal cell models. For example, the garlic by-product ajoene was found to initiate protein kinase C-δ (PKCδ)-mediated activation of Nrf2 signaling in the human hepatoma cell line HepG2 [337]. Three other garlic organosulfur compounds, diallyl sulfide, diallyl disulfide, and diallyl trisulfide, were shown to activate the Nrf2 antioxidant response via a PKC-independent mechanism in the same cell line [338].

Curcumin is a very potent activator of the Nrf2 antioxidant response in multiple cell types. One group reported that curcumin induced an increase in HO-1 levels in renal epithelial cells via activation of the Nrf2/ARE pathway [339]. In another study, liver and small intestine collected from wild-type and Nrf2 knockout mice treated with curcumin (1000 mg/kg) were analyzed to generate a global gene expression profile. The data revealed that curcumin modulated the expression of 822 and 222 Nrf2-regulated genes in the liver and small intestine, respectively, including detoxification enzymes such as HO-1, GST, and thioredoxin-interacting protein [339]. Curcumin was also shown to activate the Nrf2 response in human monocytes in a protein kinase C delta (PKC)-dependent manner [340]. Multiple lines of evidence suggest that the neuroprotective properties of curcumin may involve Nrf2 activation. Rats subjected to permanent focal cerebral ischemia exhibited neurological deficits that were reduced in a curcumin-treated group, potentially as a result of Nrf2 and HO-1 up-regulation [341]. Curcumin was also shown to induce Nrf2 nuclear translocation, expression of

Nrf2 target genes (e.g., the gene encoding HO-1), and up-regulation of GSH and glutathione disulfide in primary cerebellar granule neurons [342]. Data from another study revealed that curcumin induced an increase in HO-1 and NQO1 expression levels, alleviated oxidative stress, and preserved mitochondrial potential in H_2O_2-treated spinal cord astrocytes prepared from $Nrf2^{+/+}$ but not $Nrf2^{-/-}$ mice [343].

The activation of Nrf2 signaling by polyphenols and organosulfur compounds could occur via multiple mechanisms. One mechanism involves the participation of these agents in redox cycling reactions (e.g., quinone oxidation/reduction reactions) that lead to the formation of ROS, which in turn oxidize key regulatory cysteine residues of Keap1 (Fig. 18.9) [308,312,313,344]. Alternatively, polyphenols and organosulfur compounds can themselves covalently modify the Keap1 cysteine residues. Both mechanisms should result in a disruption of the Nrf2−KEAP-1 interaction, thereby leading to Nrf2 stabilization and activation [312,345]. Because Nrf2 is degraded by the ubiquitin proteasome system (UPS), the activation of Nrf2-mediated transcription by polyphenols and organosulfur compounds could also occur via UPS inhibition (Fig. 18.9) [346−351].

B Stimulation of PGC-1α-Mediated Mitochondrial Biogenesis

PGC-1α is a transcriptional co-activator that forms a complex with several transcription factors including PPARγ, Nrf1/2, and estrogen-related receptors [41,352,353]. The PGC-1α-dependent transcriptional network plays a central role in regulating mitochondrial biogenesis and cellular energy homeostasis [39−41]. In addition, PGC-1α is up-regulated in cells exposed to an oxidizing insult and is involved in regulating the cellular antioxidant response by inducing the expression of ROS-detoxifying enzymes (Fig. 18.9) [354]. Several groups have shown that PGC-1α carries out a protective function in cellular and animal models relevant to PD. PGC-1α-null mice are sensitized to nigral dopaminergic cell death and oxidative damage elicited by MPTP [354], and nigral neurons from these animals are more vulnerable to degeneration resulting from aSyn over-expression [355]. Genes regulated by PGC-1α are down-regulated in PD brain, and PGC-1α expression alleviates dopaminergic cell death in primary midbrain cultures exposed to rotenone or aSyn-encoding virus [356], although other studies have revealed that prolonged PGC-1α over-expression can lead to a depletion of striatal dopamine in rodent midbrain [357,358]. Collectively, these observations suggest that small molecules or botanical extracts that rescue the down-regulation of PGC-1α triggered by PD-related insults by restoring PGC-1α expression up to (but not in excess of) basal levels could potentially slow neurodegeneration in the brains of PD patients.

A number of polyphenol-enriched extracts and individual polyphenols have been shown to enhance neuronal health by promoting mitochondrial biogenesis and cellular energy balance. In one study, an extract prepared from flowers of the elderberry tree and one of its polyphenolic constituents, naringenin, was found to activate PGC-1α in mouse embryonic fibroblasts [359]. A number of groups have reported that resveratrol stimulates mitochondrial biogenesis and induces an increase in the expression of genes involved in this process in cellular and animal models [281,360−363]. Mitochondrial biogenesis was also enhanced in brain and muscle tissues of mice fed a quercetin-rich diet compared to control animals [364]. Additional evidence suggests that various isoflavones, including genistein, biochanin A, and formononetin, promote mitochondrial biogenesis [365]. The stimulatory effects of plant polyphenols on mitochondrial biogenesis may be mediated by the up-regulation or activation of SIRT1, an enzyme that stimulates PGC-1α activity by catalyzing its deacetylation [361,364,366].

In addition to stimulating the function of PGC-1α, some plant extracts and polyphenolic constituents can apparently ameliorate toxin-induced mitochondrial dysfunction by directly interacting with components of the electron transport chain (Fig. 18.9). This direct interaction could be an effective way to interfere with an early stage of oxidative stress—namely, ROS formation associated with mitochondrial dysfunction. Consistent with this idea, quercetin, kaempferol, and epicatechin have been shown to inhibit mitochondrial H_2O_2 production triggered by rotenone, potentially by competing with rotenone for the hydrophobic pocket of complex I [124].

C Alleviation of Glial Activation

Evidence suggests that polyphenols protect against neurotoxicity by alleviating neuroinflammation elicited by the activation of glial cells [367,368]. Multiple groups have reported that polyphenol-rich extracts and individual polyphenols such as ANCs, stilbenes, apigenin A (a flavone), and chlorogenic acid (a phenolic acid) attenuate the up-regulation of pro-inflammatory agents, including cyclooxygenase-2 (COX-2), NOS, NO, TNF-α, and IL-1β, in RAW 264.7 macrophages or BV-2 microglial cells activated with LPS [266,369−374]. In some of these studies, it was demonstrated that the polyphenols carried out their antiinflammatory effects by inhibiting the LPS-dependent activation of NF-κB, a transcription factor involved in mediating the inflammatory response [369,370,372,374]. Resveratrol was shown to alleviate neurodegeneration in the 6-OHDA rat model of PD by

reducing the expression of COX-2 and TNF-α in the SNpc [262]. It was also reported that isoflavones from *T. pratense* alleviate LPS-mediated microglial activation and the production of TNF-α, NO, and superoxide in neuron-glia cultures and microglia-enriched cultures from rat midbrain [285].

VIII CONCLUSIONS AND FUTURE DIRECTIONS

From this overview, it is evident that there have been remarkable advances over the past 20 years in our understanding of neuroprotective effects of dietary phytochemicals in AD and PD. Plant constituents that have been characterized in terms of their ability to alleviate neurodegeneration in cellular and animal models of AD or PD include isothiocyanates (found in cruciferous vegetables), sulfur compounds (found in garlic), and polyphenols (including flavonoids and nonflavonoids found in a variety of fruits, vegetables, nuts, and cereals). Mechanisms by which these phytochemicals are thought to carry out their neuroprotective effects include activation of cellular antioxidant responses mediated by the transcription factors Nrf2 and PGC-1α, stimulation of PGC-1α-mediated mitochondrial biogenesis, and attenuation of glial activation. A number of dietary plant constituents, particularly polyphenols, are associated with reduced AD or PD risk and have been shown to penetrate the BBB, suggesting that they could be used as therapies in patients.

A goal for future research will be to achieve a better understanding of which types of phytochemicals have the highest neuroprotective activity in AD or PD models. In a recent study, extracts enriched with ANC and/or PAC were found to have a greater propensity to alleviate rotenone-induced neurotoxicity in primary midbrain cultures compared to extracts containing stilbenes or phenolic acids [266]. Another goal will be to better understand the extent to which the neuroprotective effects of complex botanical extracts are mediated by synergies among multiple constituents, as opposed to activities of individual chemical entities [375–379]. It will also be critical to develop formulations and semisynthetic derivatives of plant constituents with improved brain bioavailability and neuroprotective activity. For example, a number of innovative methods are being used to enhance the BBB penetration of weakly bioavailable polyphenols such as resveratrol and curcumin, including nanoparticle encapsulation [380–382]. Another focus will be to assess the role of the gut microbiome in modulating neuroprotective effects of plant constituents. For example, a recent study revealed that 3-hydroxybenzoic acid and 3-(3′-hydroxyphenyl)propionic acid, two phenolic acids generated by microbiota-mediated metabolism of anthocyanidins in grape-seed extract, accumulate in rat brain and interfere with Aβ aggregation [383]. Ultimately, the insights from these studies, coupled with advances in the ability to diagnose AD and PD at earlier stages via biomarker analysis [384,385], will set the stage for developing phytochemical-based intervention strategies to delay disease onset or slow neurodegeneration in the brains of patients.

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Chapter 19

Diet and Supplements in the Prevention and Treatment of Eye Diseases

Julie A. Mares[1], Amy E. Millen[2], Thomas P. Lawler[1] and Courtney K. Blomme[1]

[1]*University of Wisconsin-Madison, Madison, WI, United States,* [2]*University at Buffalo, The State University of New York, Buffalo, NY, United States*

I INTRODUCTION

The existence of ocular manifestations of nutrient deficiencies has been well known for more than a century and is well-described. The reader is referred to other texts and reviews that describe the role of nutrition in other aspects of vision that are briefly summarized in the following paragraphs. The role of vitamin A in preventing night blindness and xerophthalmia, which are common problems in developing countries, has been widely discussed [1]. Food shortages, as occurred in Cuba in 1991–94 and among the allied prisoners of World War II, or chronic alcohol use can result in a condition broadly referred to as nutritional amblyopia, which results in blurred vision and reduced visual acuity (recently discussed in Ref. [2]). This may be the result of poor intake and absorption of B vitamins or antioxidants, alcohol and tobacco toxicity, or a combination of these factors.

More recently, it has become apparent that nutrition may be important to some patients with hereditary visual disorders. One of the more common conditions is retinitis pigmentosa, an autosomal dominant condition resulting in progressive visual loss. It begins with the loss of night vision in childhood or adolescence. This is followed by the loss of peripheral vision because of the degeneration of rods, and finally the loss of central vision because of the degeneration of cones (recently reviewed in Ref. [3]). Vitamin A supplements have improved some aspects of retinal function in patients with retinitis pigmentosa, but vitamin E had an adverse effect [4]. Subsequent research indicated that supplementation with lutein [5] or docosahexaenoic acid (DHA) [6] (in addition to vitamin A) slowed the rates of retina degeneration and visual decline, but it may be that adequate intakes through dietary means are effective and may pose less risk over the long term [7].

Nutrition may also be important to the development of the visual system in newborns. Some studies, but not all, have observed better visual development in infants who were breast-fed, as opposed to those who were bottle-fed. This has led to a search for the nutritional differences between breast milk and infant formulas that may explain better vision in breast-fed infants. In two recent studies, breastfeeding was associated with better visual acuity at age 3.5 [8] and 4–6 years [9]. Breast milk contains high levels of DHA, which rapidly accumulates in retinal photoreceptor membranes neonatally. DHA supplementation has been reported in some, but not all, studies to improve visual functions in some preterm and term infants (reviewed in Ref. [10]). Some suggest that improvements may only be transient. One study reported that DHA supplementation for 6 months postnatally did not improve vision in later childhood [9]. This suggests the possibility that other components of breast milk generally missing from infant formulas, such as carotenoids, may be responsible for better vision in breast-fed infants. Considerable evidence in the past two decades indicates a unique role for specific dietary carotenoids in eye health throughout the lifespan. This is discussed in the next section. Overall nutritional intake in infancy, childhood, and adolescence might influence chronic age-related eye diseases that develop in later life. Evidence suggests that childhood diet influences the risk of cardiovascular disease later in life [11], but the influence on age-related eye diseases, which are the focus of this chapter, has not been investigated.

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The impact of nutrition on age-related declines in vision and age-related eye disease has been investigated over only the past 30 years. The deterioration of human vision advances with age. Over 80% of blindness worldwide occurs in people over age 50. This chapter addresses the influence of diet on the most common causes of vision loss in middle-aged and older people: age-related cataract, age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy (DR).

The aging public's awareness of the decline in vision with age, and of the possibility that nutrition may influence this decline, has driven the marketing of nutritional supplements, which are sometimes costly and of uncertain benefit. In this chapter, we consider the existing evidence for the benefits of certain diets and supplements in slowing age-related visual problems associated with cataracts, AMD, DR, and glaucoma.

II LUTEIN, ZEAXANTHIN, AND EYE HEALTH THROUGHOUT THE LIFESPAN

There is evidence to suggest that the eye may uniquely require three oxygenated carotenoids for good vision throughout life, as well as to limit degeneration of the retina and lens of the eye, which contributes to AMD and cataracts in later life. These include lutein (L), zeaxanthin (Z), and L metabolite, meso-Z (MZ). These carotenoids cannot be cleaved to vitamin A and have not been considered essential for life, growth, and reproduction (the criteria that typically defines essential nutrients). However, they may be uniquely important to the eye and essential for optimal vision in the young and aging. The reader is referred to recent comprehensive reviews [12–14]. Overall, the evidence is limited, but suggestive and actively under investigation. Below, an overview of these carotenoids is given and their possible role in vision throughout life is described. In Sections II–IV, the evidence that suggests their importance in slowing age-related cataract, macular degeneration, glaucoma, and DR is discussed.

L, Z, and MZ, together, are the most abundant carotenoids in the eye and are selectively concentrated in the macula and most other ocular tissues, to the exclusion of [15–17] other carotenoids which are found common in human blood and tissues. Exceptions are in the retinal pigment epithelium (RPE) of the eye (the blood–retina barrier between the blood vessels that nourish the back of the eye and the rod and cone photoreceptors), and ciliary body (part of the layer of tissue (uvea) that delivers oxygen and nutrients to other areas of the eye) [15]. Monkeys deprived of plant foods do not have these carotenoids L, Z, and MZ in their eyes, demonstrating that obtaining them from the diet is essential [18,19]. In humans, their concentration [15,17] and optically measured density [20,21] in the macular area of the retina is highly variable. The accumulation of these carotenoids is referred to as macular pigment (MP). It is absent in premature human infants [22], is low in autopsy samples of newborn infants, and is higher in samples from older infants and children up to 4 years of age [17]. Thus, the current evidence suggests that L, Z, and MZ accumulate in the retina rapidly during late gestation and infancy, if supplied by the mother's diet or, after birth, by breast milk or infant formulas containing these carotenoids.

Historically, breast milk has been the primary source of L and Z after birth. Consistent with the exclusive accumulation of L and Z in the macula in early life, breast milk appears to selectively concentrate these carotenoids, especially in the first few months of lactation. In fact, L and Z are proportionally more prevalent in breast milk than maternal serum [23,24]. In contrast to the carotenoid content of breast milk, carotenoids are not routinely added to infant formulas. Research is needed to determine immediate and long-term impact of breast feeding on vision, compared with feeding infant formulas which lack these carotenoids and appear to be less bioavailable than breast milk [25] when added to infant formulas. Additionally, more work needs to be done to determine the level of carotenoids that should be included in infant formula for infants who do not receive breast milk. Lastly, the need for these carotenoids might be particularly high in premature infants who do not have the benefit of optimally accumulating carotenoids in utero and are limited in ability to obtain them after birth. Until more is known, it is prudent to provide infants with early life nutrition such as can be provided by breast feeding from mothers with adequate intake of these carotenoids.

L and Z accumulate most markedly in the center of the macula. The timing of their accumulation and the relative proportion of L, Z, and MZ corresponds to the maturation of the macula over the first 4 years of life (recently reviewed in Ref. [26]). The time period of the accumulation of L and Z in the retina also corresponds to the accumulation of long-chain polyunsaturated fatty acid (LC PUFA), arachidonic acids (synthesized from essential fatty acid, linoleic acid) and DHA (synthesized from alpha-linolenic acid), which are also highly concentrated in retinal tissue (like the brain and other neural tissues (reviewed in Ref. [27])). These xanthophyll carotenoids and omega-3 fatty acids appear to be essential for the development and/or maintenance of normal RPE cells [28], the layer of cells that support the nutrient and metabolic needs of the rod and cone photoreceptors.

L, Z, and MZ in the macula (and L and Z in the lens) comprise is commonly referred to as "macular pigment."

These carotenoids absorb short wavelength (about 400−530 nm) light in the blue range of the spectrum that is known to damage photoreceptors and the RPE. In monkeys, depletion of L and Z in early life results in increased susceptibility to blue-light damage [29]. Preterm human infants supplemented with L had greater sensitivity responses of rod photoreceptors [30]. However, the extent to which a lack of carotenoids in early life creates lasting visual advantages or prevents lifelong vision problems is unknown. Vision throughout life might also be modified by the degree to which L and Z have accumulated in utero and by the intake of foods containing these carotenoids in later infancy and childhood. Supplementation of carotenoid-depleted monkeys reduced blue-light damage to the fovea [29].

It has long been proposed and recently demonstrated that these carotenoids enhance vision function. This was recently reviewed in Ref. [12]. Briefly, supplementation with L, Z, and/or MZ for 3 months to 3 years has improved visual acuity and or contrast sensitivity in many, but not all, clinical trials. Visual acuity improvements were noted in individuals with AMD or DR, but not, to date, in individuals with healthy vision. Differently, improvements in contrast sensitivity have been observed in samples of both healthy men and women and in individuals with diabetes or early- and late-stage AMD. Improvements in the time it takes to recover from bright lights (photostress recovery) or enhancement in the ability to see in conditions of glare have been observed in a few studies, but not most. Improvements in visual processing speed and rates of dark adaptation with adequate L and Z intake have been hypothesized and are supported by limited data, but have not been well-studied. Supplementation with L and Z slowed vision loss in adults with retinitis pigmentosa who were also taking vitamin A [5]. The ability to accumulate macular pigment is highly variable, particularly over a few months. The improvement in trials is often the largest in people who have made the most marked improvements of MP optical density levels. Also, improvements in vision outcomes have been more consistently observed when supplements include other antioxidants and/or long-chain polyunsaturated omega-3 fatty acids (LC omega-3 PUFAs).

The density of L and Z in the lens and retina may influence the eye diseases that occur in later life. It has been reasoned [31] that infant eyes are particularly vulnerable to oxidative damage in early life, which may have consequences decades later. Because of the redundancy of the visual system it may be difficult to observe the impact of early life nutrition on vision function in later life in small samples of people.

Sources of L and Z are also important from childhood through adulthood. Food sources of L and Z include a wide variety of fruits and vegetables, grains, and eggs [32]. They are particularly concentrated in green leafy vegetables, but corn products and eggs may comprise significant sources for some people, such as American Hispanics. Mean intake levels estimated in the 2011−12 National Health and Nutrition Examination Survey (NHANES) were 1.7 mg/day in adults over 20 years of age and 0.8 mg/day in children 2−19 years of age [33]. Levels of L and Z intake in some South Pacific study samples are higher [34] and reached 25 mg/day in one study in Fiji [35]. Increasing obesity in the United States might contribute to reductions in carotenoid status, as obesity (and other phenotypes related to metabolic syndrome) is associated with lower levels of L and Z in serum and MP after accounting for dietary intake [20]. It is hypothesized that obesity reflects genetic and lifestyle factors which lower the absorption of these oxygenated carotenoids and their uptake and stabilization in the eye [20,36].

III CATARACT

Cataract is the leading cause of blindness worldwide, accounting for almost half of all blindness [37]. The visual burden of cataract is largest in developing countries, where malnutrition is more common and the relatively simple surgical excision of cataract is less available. However, the economic burden of cataract in developed countries is high. For example, in the United States, the total direct medical cost of cataract was $6.8 billion in 2004, representing 42% of the total direct medical cost of all visual disorders in that year [38]. Seventeen percent of Americans older than 40 years have cataract in either eye, and 5% have had cataracts extracted [39]. The rate of cataract and extraction steeply increases with age. More than half of individuals over age 80 years have had cataract surgery (United States). Lens opacities can make seeing more difficult, especially under dim light and in the presence of glare (such as when driving at night), even before surgery is indicated. Over 30 years ago it was estimated that if preventive measures could delay cataract by only 10 years, the visual and surgical burden would be cut in half [40].

Lens opacities form as a result of protein aggregation or lens fiber disorganization due to either acute metabolic insults or the gradual accumulation of damage with age. The lens must remain clear in order to collect light and focus it on the retina. Lens opacities scatter light, blurring vision. With aging, the lens also slowly becomes brunescent, adding a brown tint to vision. When light transmission is blocked substantially enough to reduce visual acuity, a cataract exists; if opacities become severe, blindness can occur.

Cataracts generally occur in three regions of the lens (recently reviewed in Ref. [41]) (Fig. 19.1). The most common region is opacities in the nucleus, or center, of the lens. This is called a nuclear cataract, and most people develop this with age. Nuclear cataract may reflect cumulative insults that have occurred since early childhood.

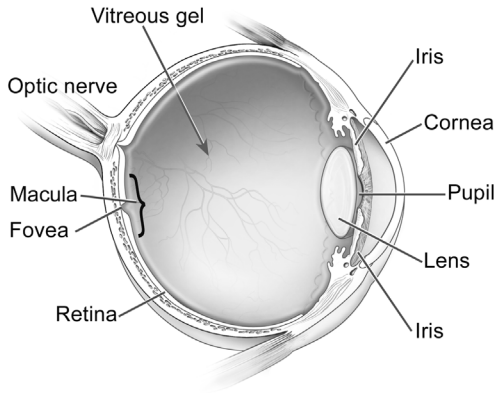

FIGURE 19.1 Anatomical features of the human eye. *Courtesy of National Eye Institute, National Institutes of Health.*

Nuclear lens fibers do not have mitochondria, nuclei, or other cytoplasmic organelles and thus lack the capacity to repair damage over one's lifetime. Less commonly, opacities develop in the region just under the lens capsule that separates the lens from the vitreous humor (posterior subcapsular cataracts, or PSCs), and in the fiber layer between the outside edges of the lens and the nucleus (cortical cataract). Cataracts sometimes occur exclusively in one region However, often opacities occur in multiple regions, particularly as cataracts become more severe.

A Causes

The pathogenesis of cataract involves the accumulated stresses caused by physiological and environmental stressors that arise from such things as the photochemical formation of free radicals and osmotic imbalances. Most cataracts are the result of aging, but cataracts can also occur as a result of surgical or other eye trauma, steroid use, or some types of radiation. Smoking is the most common modifiable risk factor for age-related cataract in population studies [42]. Exposure to smoke from combustion of wood or other fuels [43,44] may similarly increase cataract risk. Other nonnutritional risk factors include prolonged exposure to ultraviolet (UV)-B light (particularly in the cortical region of the lens), diabetes (especially for cortical cataract and sometimes nuclear cataract and PSC), obesity, heavy use of alcohol or elevated markers of systemic and local inflammation [45] or arthritis (as a marker of inflammation) [46]. Genetic factors have been most extensively investigated for nuclear cataract, and heritability studies suggest a substantial role of genetic factors in the development of nuclear cataract occurrence and progression, but specific genotypes are not well-studied [47].

Animal studies have indicated that cataract can be induced by high-galactose diets, experimental diabetes, nutrient deficiencies (riboflavin, calcium, zinc, and selenium), or nutrient excesses (selenium) (reviewed previously in Ref. [48]). Genetic mutations or polymorphisms that cause perturbations in calcium [49] or iron metabolism [50] have also been associated with cataracts. Otherwise, in the absence of extreme metabolic insults or deficiencies, lens opacities often develop slowly over many years. A gradual influence of nutrition on the development of cataract over adult life is suspected and is supported by animal studies. In Emory mice, a mouse strain that develops cataracts in adulthood, calorie restriction slows the development of lens opacities [51]. Since the 1980s, a body of evidence has been accumulated to indicate lower rates of cataracts among populations of people who eat micronutrient-rich diets or take multivitamins (summarized in Table 19.1 and discussed later).

B Healthy Diet Patterns

Generally, healthy diets or vegetarian diets have been associated with lower risk of cataract extraction or nuclear cataract in several studies in populations around the world [51–55]. The reduced risk associated with healthy diets in epidemiological studies of generally well-nourished populations [53,54] is about twofold and not explained by single nutrients [56], suggesting additional unknown food components that may lower risk and/or that food components work jointly to lower risk. The following sections consider many specific food components which may be responsible.

1 Carbohydrates

In experimental animals, cataracts are easily developed by feeding monosaccharide-rich diets or agents that promote diabetes (reviewed in Ref. [48]). Two mechanisms for cataract promotion within the lens are proposed. When the metabolic pathways to utilize sugars are overwhelmed, sugar alcohols are formed by aldose reductase, accumulate in the lens, and can cause osmotic cataracts. Another mechanism might involve nonenzymatic glycosylation and the accumulation of advanced glycation end products in the lens [57] or cataracts [58].

Humans with galactosemia, a rare, autosomal recessive disorder leading to an inability to break down dietary galactose, as found in milk, can be treated with a galactose-free diet. However, treated individuals are still at higher risk of developing cataract throughout life [59]. In a meta-analysis of human observational studies, higher dietary carbohydrate quantity and glycemic index were associated with the risk of cortical and nuclear cataracts, respectively [60]. In people with diabetes, poor glycemic control has been associated with higher lens density [61]. The prediabetic state has also been associated with cataract

TABLE 19.1 Summary of Evidence Relating Nutritional Exposures to Cataract

Nutritional Exposure	Strength of Evidence	Comment
Healthy Diet Patterns	*Benefit of following micronutrient-rich diet patterns is likely*: A large body of evidence suggests that healthy diets lower chronic diseases and their risk factors. Four observational studies suggest lower rates of cataract among people reporting the intake of healthy diet patterns.	Two population studies suggest that the benefit of healthy diets on lowering risk of nuclear cataract is stronger than the benefit of high intake of single nutrients. Many studies in animals and humans support the benefit of numerous specific micronutrients and phytochemicals.
Carbohydrate	*Possible increased risk associated with high levels of specific or overall refined carbohydrates:* Animal studies suggest several mechanisms. A meta-analysis of population studies suggests that higher dietary carbohydrate quantity and glycemic index was associated with the risk of cortical and nuclear cataract, respectively.	Results in population studies might reflect an influence of diabetes on cataract, rather than carbohydrates specifically and/or that high-carbohydrate diets are often nutrient-poor.
Antioxidants	*Benefit of food antioxidants is likely; supplemental antioxidants do not consistently lower risk and, there is the possibility that they increase risk in some cases:* Animal studies prove that oxidative stress leads to lens opacities and that antioxidants lower indicators of oxidative stress and/or damage. Population studies in many samples indicate lower risk of cataract with higher intake or blood levels of various antioxidants. The data are most consistent for diets rich in lutein and zeaxanthin.	In population studies, diets rich in specific antioxidant nutrients are likely to be markers for diets rich in plant foods (fruits, vegetables, whole grains) which contribute a wide range of nutritive and nonnutritive antioxidants. Clinical trials of high-dose antioxidants do not generally support the benefit of one or two specific antioxidant nutrients or a combination of high-dose antioxidants. In a meta-analysis of clinical trials, high-dose beta-carotene and/or vitamin E increased mortality.
Lead	*Exposure possibly increases risk:* Two observational studies suggest that intake of antioxidants in supplements increase the risk of cataract. This risk factor and the influence of other heavy metals, particularly when dietary antioxidants are low, require further research.	
B vitamins	*Benefit of dietary riboflavin and niacin in malnourished or healthy populations is possible:* Results of one clinical trial and many observational studies suggest protective relationships with the intake of these B vitamins. *High-dose intake of folate, B6 or B12 may increase risk:* Supplemental intake of a combination of these three increased the risk of cataract extraction in one trial. Dietary folate intake in a well-nourished study sample, in the time period after folate fortification, increased the risk of PSC over 9 years.	Considered together, the evidence for an adverse effect is strongest for folate in supplements or in fortified foods, but additional research is needed to better evaluate this possibility.
Vitamin D	*The benefit of good vitamin D status (from adequate sunlight, foods, and/or supplements) on cataract is unknown.* Animal and cell studies suggest antiinflammatory properties of vitamin D. Results of three observational studies indicate lower prevalence of cataract in persons with high levels of a biomarker of vitamin D status in the blood. Relationships of vitamin D intake from foods and supplements in relation to cataract are limited to four cross-sectional studies and are inconsistent.	
Multivitamin Supplements	*Both benefit and harm are possible:* Benefit may exist for persons at risk for nuclear cataract, but multivitamins might increase the risk of cortical of PSC.	

[62]. However, the specific impact of simple sugar consumption on cataract risk is uncertain. Epidemiological associations might reflect, to some extent, broader nutrient-poor diet patterns that often accompany diets high in carbohydrates or the impact of these diet patterns or diabetes on oxidative stress or inflammation.

Animal studies suggest that other aspects of the diet might modify cataractogenesis that develops as a result of diabetes- or galactosemia-induced cataract. The development of these cataracts has been lessened by intake of plant extracts [63] or a wide variety of nutrients and phytochemicals such as vitamins C and E (previously reviewed in Ref. [64]), soy isoflavones [65], caffeine [66], resveratrol [67], and cumin [68].

2 Antioxidants

It is well known that oxidative stress increases lens damage [41]. Relationships of antioxidant nutrients to cataract development in animals, and to the occurrence of cataract in populations, have been extensively reviewed [44,48,69–71]. Deficiencies of riboflavin, selenium, and zinc—cofactors for enzymes that play important roles in oxidative defense—cause cataracts in some species (previously reviewed in Ref. [48]). Deficiencies of vitamins C or E and major water and lipid-soluble antioxidants have not been reported to cause cataracts independently, but they do protect against oxidative damage in a variety of animal and cell systems. Vitamin C is abundant in human lenses, and its levels in the lens reflect those in the diet [72]. Lipid-soluble antioxidants in lenses include vitamin E and the specific carotenoids L and Z [16], which uniquely accumulate over other blood carotenoids. Both have been demonstrated to protect against UV-light-induced peroxidation in lens cells [73].

High levels of combined antioxidants in diet or serum [74–77], or of single antioxidant nutrient in diet and blood, have been associated with lower prevalence or incidence of cataracts or cataract extraction associations with numerous observational studies [44,71,78]. In contrast, there has been no evidence in randomized clinical trials for up to twelve years, that supplementation with one or more antioxidant nutrients (above recommended dietary allowances) slow the progression of age-related cataract [79]. An exception is in one trial in which they were accompanied by a multivitamin [80]. Moreover, taking vitamin C supplements was associated with an *increased* risk of cataract extraction over 10 years in women [81]. Both high-dose vitamin C and E were associated with higher risk of cataract over about 8 years in Swedish men, across many subgroups and risk increased among long-term users [82]. One explanation for these conflicting pieces of evidence is that both deficiencies and excess of antioxidants could be harmful. In the lens, vitamin C can enhance oxidative stress and contribute to glycation of lens proteins [83,84]. Moreover, there is evidence that oxidation of vitamin C can produce a compound that enhances photosensitivity which could further promote cataracts [85]. In addition, in a meta-analysis of trials, beta-carotene and vitamin E, singly or combined, significantly increased mortality [86]. For these reasons, caution is suggested in the consumption of high-dose antioxidants for the prevention or slowing of cataracts [79].

Supplements containing L and Z have only been available since about 1995, and they are likely to become more common since the addition of L and Z to high-dose antioxidants was recommended as a standard of care in treating intermediate or advanced stages of AMD (see Section III). There is mounting evidence that intake of these carotenoids may explain, in part, the lower cataract risk associated with people who eat diets rich in vegetables. Diets and serum rich in L and Z are consistently related to lower incidence or prevalence of nuclear cataract or cataract extraction in longitudinal studies [75,87–91], except in well-nourished men and women over 60 years of age participating in a clinical trial of high-dose antioxidants [92]. However, foods rich in these carotenoids, such as green vegetables and dark green leafy vegetables, are rich in many micronutrients and antioxidants, so that consistency of this association may simply reflect micronutrient and antioxidant-rich diets in general. In one large 5-year randomized trial in people with AMD, adding L to high-dose antioxidant supplements slowed the progression to cataract surgery among participants in the lowest quintile for levels of these carotenoids in their diets, although there was no protective benefit in the larger group of participants [93]. Relationships between high-dose L and Z supplement use and general health and mortality have not been well-studied.

Thus, the overall body of evidence from animal and population studies suggests that antioxidant components of the diet may contribute to protection against cataract development. However, there is little evidence to suggest that short-term supplementation with one or a few antioxidants is likely to have an important impact on the development of cataracts, which develop over many years and are influenced by a wide variety of dietary, health, and lifestyle factors. Moreover, adverse effects on cataract development or other aspects of health are possible when taken in high doses.

3 Minerals and Heavy Metals

Research on the benefits of antioxidants has not considered the potential larger importance that antioxidant protection might have under conditions of high oxidative stress. Oxidative stress can also result from genotypes

that result in iron overload [50]. Mechanisms for toxicity of heavy metals may include depleting cellular glutathione and/or heavy metals displacing zinc and copper on enzymes involved in protection against free radicals, causing oxidative stress [96].

Only a few epidemiological studies have examined the joint effect of low intake of antioxidant-rich foods and heavy metal exposure. In a sample of fish-eating people living in an area of the Amazon, with among the highest levels of mercury exposure in the world, joint existence of poor plasma selenium and high blood mercury is associated with a dramatically higher (16-fold) prevalence of age-related cataract [95]. In one clinical trial, beta-carotene was protective against cataract only in smokers who were likely to have higher exposure to heavy metals [97]. Thus, a benefit of food antioxidants may be greater in smokers or people who are exposed to industrial pollutants.

4 Other Vitamins

B vitamins: Results of several observational studies [75,92,98–103], and one trial in a malnourished population [104], have suggested protection against the development of lens opacities in individuals with higher dietary or blood levels of the B vitamins riboflavin, thiamin, and/or niacin. Riboflavin and niacin have roles in enzymatic mechanisms to protect against oxidative stress. Supplementation with these in an undernourished population in China protected them against the occurrence of nuclear cataracts [104].

In contrast, supplementation with a combination of B vitamins (vitamin B6, B12, and folate), at doses exceeding the recommended dietary allowances, increased the risk of cataract in U.S. physicians over 7 years [105]. Consistent with this finding, in participants already using multivitamins, high dietary folate was associated with higher risk of mild PSC among participants in the Age-Related Eye Disease Study (AREDS) [92]; cataracts in the PSC region of the lens was increased twofold [106].

Vitamin D: A role for vitamin D in the functioning or health of the lens is suggested by the evidence of the vitamin D receptor (VDR) in human lens epithelium [107]. As previously reviewed, inflammation is implicated in the development of cataract and the antiinflammatory properties of vitamin D [108] have been hypothesized to protect against age-related cataract.

Three investigations have recently described the lower prevalence of cataracts among people with high, compared with low, blood concentrations of 25-hydroxyvitamin D (25(OH)D) and cataract. Serum 25(OH)D reflects intake of vitamin D from foods, supplements, and the endogenous production of vitamin D that occurs upon exposure of skin to UV-B radiation in sunlight [109]. In 1988, results of an exploratory analysis of 112 participants, indicated a decreased odds of any and cortical cataract among those with high compared to low 25(OH)D concentrations [74]. Since then, data from two cross-sectional studies, one in a cohort of postmenopausal women [110] and the other in a population-based survey from Korea [111], have suggested possible protective associations between serum 25(OH)D and cataract, but only in subsets of their samples (e.g., postmenopausal women age <70 years [110] and in men [111]). The conflicting risks of sunlight—which may increase risk of cataract—and dermal production of vitamin D—which may decrease risk for cataract—make observational associations of vitamin D status and cataract exceptionally difficult to disentangle.

There have been a small number of studies examining relationships between the intake of vitamin D from foods and or supplements and the evidence is conflicting and does not take into account intraindividual differences in the level of vitamin D synthesized in the skin on exposure to sunlight or the competing risk of sunlight. Food sources of vitamin D include naturally occurring sources like fatty fish, fish liver oil, mushrooms, and egg yolk as well as fortified food sources such as milk, orange juice, breakfast cereals, and margarine [109]. A protective association of prevalent nuclear cataract with retrospectively recalled milk consumption [75] may reflect, in part, vitamin D intake or it could reflect a protective association of riboflavin, also associated with lower odds for nuclear cataract, or it could reflect an overall dietary pattern. Studies examining associations between cataract and estimated intake of vitamin D from all foods consumed reported null associations [52,101]. The evidence to describe relationships between the intake of vitamin D in supplements and cataract is limited to one retrospective study in which the intake of vitamin D from supplements was associated with increased odds of nuclear and cortical cataracts [112]. However, the confounding effects of other supplemental nutrients and lifestyle factors could not be accounted for.

In summary, study of the association between vitamin D status and cataract needs additional research in order for any conclusions to be drawn. Ideally, future studies will concurrently evaluate vitamin D intake from all sources and the competing risk of excess ocular sunlight exposure which may accompany higher rates of vitamin D synthesis in the skin. The role of vitamin D status in the eye is likely to vary by anatomical site and the extent to which vitamin D may influence the health of the cornea, lens, or retina differently is still under study.

5 Multivitamin Supplements

In contrast to the evidence for high-dose antioxidants and cataract, a large body of evidence from observational

studies [80,113–116] and clinical trials [104,106,114] indicate a protective effect of multivitamin use on nuclear cataract. However, evidence does not consistently support a benefit for cortical or PSC opacities. In one 9-year trial, the benefit of multivitamins was not observed for cortical opacities and the risk of cataracts in the PSC region of the lens was increased twofold [106].

IV AGE-RELATED MACULAR DEGENERATION

A Overview

AMD is a result of deterioration of the macula (see Fig. 19.1), the cone photoreceptor-rich part of the retina responsible for central vision and reading fine detail. AMD is the leading cause of blindness in developed countries and the third leading cause worldwide [37]. By the age of 65, roughly 8% of people in the United States have an intermediate form of AMD, which has a high risk of progressing to advanced AMD [117], and 2% of Americans over 80 years have advanced AMD [117]. There is no cure for this condition, and medical and surgical treatments are limited to people with one type of advanced AMD (wet AMD) with limited long-term effectiveness. Because of the steep increase in AMD with age, prevention is likely to have a large impact on the social and economic burden of this condition.

The early stages of AMD are signaled by yellowish white deposits, or drusen (Fig. 19.2), in the subretinal space and in the posterior part of the photoreceptor layer. Drusen can vary in size and area; more extensive drusen development is predictive of greater eventual progression to advanced stages. In intermediate stages, there are sometimes areas of hyper- or hypopigmentation (retinal pigment abnormalities) in the retina pigment epithelial (RPE) cells, a single layer of cells that support the rod and cone photoreceptors, caused by disturbances in the distribution of melanin pigments. Extensive drusen and retinal pigment abnormalities are thought to signal the existence of pathological processes associated with a distressed central retina. These changes are thought to compromise the ability of nutrients and oxygen to flow from the choroidal blood supply, through Bruch's membrane, to the RPE cells and the photoreceptors they support. Drusen may also reflect the existence of inflammatory processes that contribute to degradation of this area.

When deterioration of the macula proceeds to the extent that the RPE cells and the rod and cone photoreceptors they support die, advanced AMD occurs. It is sometimes referred to as "dry" AMD when it is limited to atrophy of the RPE and photoreceptors. If growth of new blood vessels occurs, then the advanced AMD is referred to as "wet" AMD. Impairment in visual acuity (after correction for glasses) is common with advanced AMD, progresses with the severity of disease, and can cause legal blindness. Bleeding or leaking of these vessels can also cause acute limits in vision. Advanced AMD of both types interfere with vision in the center of the visual field (such as that needed to view a person's face straight on) and the ability to read fine detail needed, for example, to read newspapers.

Rod-photoreceptor-related vision loss also occurs with advanced AMD, and often even before the loss of visual acuity associated with advanced AMD. There is impairment in rod-mediated dark adaptation, characterized by a slow recovery of visual sensitivity in low light conditions, such as when moving from areas of bright to dim illumination. This is thought to be caused by deposition of hydrophobic lipids in the RPE/Bruch's membrane complex with AMD and aging. This could slow the rate of delivery of vitamin A to rod photoreceptors, creating a transient local deficiency of vitamin A for rod photoreceptors

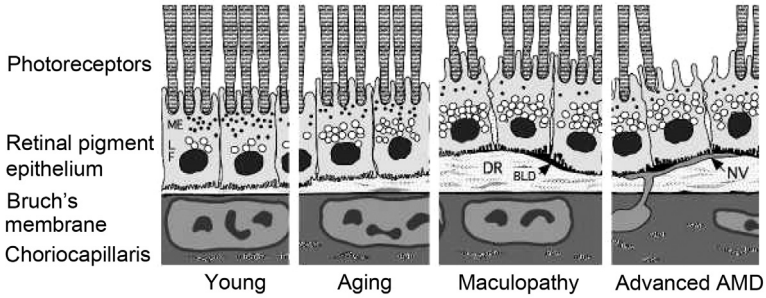

FIGURE 19.2 Changes to layers of retina in the macular region during aging, and progression of AMD in early and advanced stages. With age, there is an accumulation of lipofuscin (*LF*), a decline in melanin (*ME*) in the RPE, and a thickening and lipid enrichment of Bruch's membrane. Basal laminar deposits (*BLD*) are seen in early maculopathy but may also reflect aging. Drusen (*DR*) accumulation also characterizes early maculopathy. In intermediate AMD, *DR* becomes more extensive, and there are areas of the RPE that are hypopigmented or hyperpigmented (because of *ME* clumping). In more advanced AMD, there is atrophy of the photoreceptors and there may be neovascularization (*NV*)—the growth of new blood vessels and narrowing of the choriocapillaris. *Courtesy of Francois Delori, Schepens Eye Research Institute/Harvard Medical School, Boston, MA.*

(rods), slowing the recovery of light sensitivity by rod photoreceptors [118]. This localized vitamin A deficiency reduces the amount of vitamin A metabolite, 11-cis-retinal, available to combine with the protein, opsin, to form the visual pigment rhodopsin and the rate of phototransduction (converting light energy into neural impulses) [119]. This idea is consistent with the observation that short-term, high-dose retinol supplementation increased the rate of rod-mediated dark adaptation in people with early AMD or signs of retinal aging [120].

A significant proportion (22%) of adults over 60 years of age, in otherwise normal retinal health, have been also observed to have dark adaptation abnormalities [121], and were observed to increase the risk of developing AMD three years later [122]. This might explain common reports of problems seeing in low light conditions in people with early stages of AMD, even in persons with good visual acuity, tested at high illumination [123,124].

B Causes

AMD appears to develop as a result of a complex interplay of multiple dietary, environmental, and genetic factors that influence oxidative stress [125], inflammation [126,127], and light damage [128]. Smoking is the most commonly reported risk factor [129] for AMD in epidemiological studies. It is also commonly thought that damage by light, especially in the blue range, promotes AMD, although epidemiological studies have not consistently observed higher rates of AMD among people with high levels of sunlight exposure. This might be due to the difficulty in assessing the amount, type, and timing of sun exposure [130]. Some studies indicate high risk for AMD among people with cardiovascular disease [131,132] or risk factors such as obesity, diabetes, and hypertension [133,134].

Next to smoking, the most consistent, strong risk factor for AMD is family history [134]. People with AMD more commonly have certain variants within genes related to complement activation in the inflammatory response. While multiple complement related genes have been identified in AMD risk, the complement factor H (*CFH*) and age-related maculopathy susceptibility-2 (*ARMS2*) genes carry the greatest amount of risk. The *Y402H* variant A within *CFH* explains more than half of the population risk of AMD and is associated with 2.5−6.0 times greater AMD risk than individuals without the risk variant [135]. A variant with independent, yet similarly strong effects is the A69S variant (rs10490924) within *ARMS2* [136]. The genetic predisposition to AMD may involve a propensity for inflammation or exacerbation of an inflammatory response [126]. Some studies observe a higher prevalence of AMD among people with elevated C-reactive protein [137,138], an indicator of systemic inflammation, or among people with inflammatory diseases, such as gout [139], or who have used antiinflammatory medicines, which may signal the presence of inflammatory disease [140]. The evidence that diet and supplements may prevent or slow AMD is discussed later and summarized in Table 19.2.

C Healthy Diets

A wide body of evidence from a variety of study types supports the idea that food choices influence the development and progression of AMD, as discussed in subsequent sections. Healthy diet patterns that are rich in a wide variety of nutrients are consistently associated with lower AMD risk. Various healthy diet patterns have been related to a lower prevalence of early [141−143] and advanced AMD [142−145] and lower incidence of early AMD [146] and progression to advanced AMD [143,147].

Studies have looked at how healthy lifestyle factors, genetics, and/or diet patterns interact to contribute to AMD risk. It is unclear whether the impact of healthy diets and lifestyles is greater in people with high or low genetic risk. In one study, the reduction in the chance of having AMD was large (threefold lower) when healthy diets are jointly accompanied by moderate to high levels of physical activity and avoidance of smoking [141]. Moreover, women with a genetic predisposition for developing AMD (indicated by possessing two high-risk alleles for the *CFH Y420H* gene) significantly increased their odds of having AMD 6 years later if they had a history of heavy smoking, low scores on a healthy diet index and low levels of exercise. Results of another study in a separate sample found that higher adherence to a Mediterranean-style diet was protective in individuals who possessed at least one *nonrisk CFH Y420H* allele, but conferred no benefit in individuals with two high-risk *CFH* alleles [147]. Having high levels of body fat, which likely reflects a combination of genetic factors, diet, and physical activity, has been associated with a 12-fold higher risk for incidence of early or advanced AMD [148] and, together with smoking, has been observed to lower risk for progression of AMD 8- to 19-fold among people who had one to two copies of two common risk alleles [149]. The stronger associations observed when healthy diets, physical activity, not smoking, and genetic factors are considered jointly may be explained by the fact that they all contribute in lowering oxidative stress, inflammation, blood pressure and improving blood lipids, all of which are thought to promote AMD.

Moving forward, it might be possible to gain a better understanding of optimizing specific aspects of diet, by combining data from several large epidemiological studies and examining relationships with specific foods *within* groups of people who have similar overall diet quality.

TABLE 19.2 Summary of Evidence Relating Diet to AMD

Nutritional Exposure	Strength of Evidence	Comment
Healthy Diet Patterns	*Benefit of following micronutrient-rich diet patterns is likely:* A large body of evidence from results of studies in animals and humans support the benefit of numerous specific micronutrients and phytochemicals. Three studies suggest that scores on overall healthy diet patterns lower risk. (One study suggests risk lowering is particularly marked when combined with physical activity and not smoking.)	
High Glycemic Foods	Diets with low glycemic index scores might reduce the risk of developing AMD. Hyperglycemia in known to contribute to oxidative stress and inflammation, known to promote AMD. Results of a few observational studies suggest lower rates of AMD are associated with diets with low glycemic index scores.	A low glycemic index score is likely to be a marker for a plant- and nutrient-rich diet that contains many food components likely to lower AMD risk.
Antioxidants	*Benefit of foods or supplements rich in many antioxidants is likely*; Oxidative stress in the retina is high and known to contribute to AMD. In clinical trials, supplementing with individual antioxidants does not slow the progression of AMD. However, the benefits of a specific combination antioxidant supplement in slowing progression has been demonstrated in one large study sample (AREDS); benefits persisted over longer follow-up of the cohort for about 10 years. Benefit is also suggested by the results of several smaller studies. While observational studies suggest that antioxidant-rich diet patterns are likely to prevent AMD, antioxidant supplements have not been shown to *prevent* AMD.	*Caveats regarding AREDS-tested supplements:* – The longer-term risks and benefits are unknown. – Whether lower doses or different combinations of nutrients may have more benefit or lower long-term risk is unknown. Trials of one or two high-dose antioxidants have not shown benefit.
L and Z	*Benefit in slowing AMD and/or improving vision is likely:* The biological plausibility that L and Z can protect against oxidative stress, inflammation, and/or reduction of damage due to light exposure is strong and supported by studies in both animals and humans. In population studies, diets high in foods that contain L and Z are consistently related to lower risk for advanced AMD (although inconsistently related to lower risk for earlier stages). Several small and short-term clinical trials provide preliminary evidence to suggest that L and Z supplementation may improve vision in people with AMD. Benefit in slowing advanced AMD in individuals with low dietary intake is suggested by secondary analyses in a large clinical trial (AREDS2).	Diets rich in L and Z may reflect the overall benefit of high intakes of many micronutrient-rich foods. The ability to accumulate L and Z (and therefore, the potential benefit) varies across people. Differences in accumulation in the macula, genetic risk factors for AMD, and survival bias may make it difficult to observe consistent relationships between L and Z intake and AMD incidence and progression.
Zinc	*Benefit in slowing progression is proven:* High-dose zinc supplements slowed the progression of intermediate to advanced AMD in a large, multicenter, placebo-controlled clinical trial over 6 years; benefit was sustained over about 10 years of follow-up. In combination with antioxidant supplements, this supplement also reduced moderate visual acuity loss,	The safest dose with benefit is unknown. The long-term benefits and risks of consuming high levels of zinc in supplements are not well-studied. Inconsistency across observational studies might be explained by differences in the degree to which other components of foods rich in zinc (milk, beans, meats, and shellfish) or correlated nutrients or lifestyles have been accounted for. The benefit of zinc may depend on

(*Continued*)

TABLE 19.2 (Continued)

Nutritional Exposure	Strength of Evidence	Comment
	consistent with some other smaller studies that observed improvements if vision function with supplementation. *Benefit of adequate zinc intake from foods in slowing development of AMD is likely:* Zinc deficiency impairs retinal function in animals and humans. Diets high in zinc have been related to lower AMD in some but not all epidemiological studies.	the exposure to toxic metals from cigarette smoke and pollution.
Dietary Fat	*High intake of total fat might encourage the development of AMD:* High fat intake in mice with a genetic propensity to lipid disorder results in AMD-like lesions. Overall fat intake is associated with lower prevalence or progression of AMD in most population studies (although not always statistically significant). *Benefit of foods rich in LC omega-3 fatty acids is likely*: The retina is high LC omega-3 PUFAs which require a high rate of renewal. Diets high in omega-3 fatty acids are associated with lower inflammatory markers in the blood. Deficiency of omega-3 fatty acids in nonhuman primates increases sensitivity to blue-light damage. High intake of LC omega-3 fatty acids or fish is associated with lower risk for AMD in 7 of 8 study samples. In two randomized controlled trials of DHA and EPA supplements, added to high-dose antioxidants did not slow progression of persons with AMD over about 5 years.	Higher risk for AMD among people with diets high in fat might reflect lower overall nutrient density of high-fat diets or other aspects of lifestyle associated with the intake of high-fat diets. Diets high in LC omega-3 fats or fish may be related to lower AMD risk due to unmeasured and controlled for aspects of diet (intake of vitamin D and/or selenium) or lifestyle. Benefits should be considered in conjunction with the possibility that fish and some fish oils may contain mercury or other contaminants. Inconsistencies in protective associations in observational studies or across populations who vary in intake of omega-3 fatty acids or clinical trials might reflect modifying effects of genetic risk for AMD or the metabolism of omega-3 fatty acids.
B vitamins	Benefit is possible: Benefit is suggested by only one randomized clinical trial and two observational studies. More research is needed.	The evidence is insufficient to indicate which B vitamin(s) might be protective and whether folic acid supplements might be harmful over the long-term in some people.
Vitamin D	*Benefit of good vitamin D status (from adequate sunlight, foods, and/or supplements) is possible.* Animal and cell studies suggest antiinflammatory properties of vitamin D. In cohort studies and studies of population-based survey data, associations between 25(OH)D concentrations, as an indicator of vitamin D status, and early AMD are inconsistent. Only one study is of a prospective design, but data on vitamin D status was derived from a retrospective review of Medicare claim files and AMD diagnosis. Data from one national survey and two case-control studies suggest that an association between 25 (OH)D and late-staged AMD may exist but few robust studies have investigated this association.	Higher blood levels of vitamin D could be related to other aspects of diet or lifestyle that could protect against AMD.
Multivitamin Supplements	*Benefit is unknown:* The use of multivitamin supplements has not been associated with lower risk for AMD in population studies (except in Americans who did not report drinking milk daily). Multivitamins did not lower the incidence of AMD over 11 years in a large cohort of physicians.	The impact of multivitamins on the onset or worsening of AMD has not been tested in clinical trials.

Combining data will also allow for more statistical power to examine aspects of diet by levels of genetic risk, levels of physical activity, and other lifestyle factors.

D Glycemic Index

The glycemic index of foods was introduced to be another possible aspect of diet that could influence the development of AMD [150]. Although advanced glycation end products have been found in drusen, it is not yet known whether they are a cause or consequence of degenerative changes. Degeneration of the retinal vasculature is a well-known complication of diabetes mellitus; yet the presence of diabetes has sometimes, but not always, been related to AMD in epidemiological studies. The biological plausibility that elevation in blood sugar promotes AMD, particularly in the absence of diabetes, remains untested. Nevertheless, diets with a low glycemic index often include few refined grains and sugars and plenty of fruits, vegetables, whole grains, legumes, and milk, which have numerous components that could protect against AMD. Thus, high glycemic index diets, like high-fat diets, may be related to higher rates of AMD, in part or in whole, because they lack a wide variety of protective nutrients and other diet components.

E Antioxidants

Oxidative damage to proteins, lipids, and DNA, by free radicals within the photoreceptor outer segments (POS) or RPE, can be the result of photooxidation of lipids from light exposure. They can also be the by-product of metabolic events, such as oxidative metabolism or enzyme reactions that use oxygen (such as xanthine oxidase). The retina is particularly susceptible to oxidative stress, because of its high rates of oxidative metabolism and light exposure, and its high concentration of long-chain polyunsaturated fatty acids, whose double bonds are vulnerable. Free radicals that propagate oxidative damage may also be produced directly as a mechanism for biological defense, as is the case when white blood cells respond immunologically to pathogens with an oxidant boost.

In epidemiological studies, when associations with antioxidant nutrients are considered one at a time, low levels of one or more antioxidants in the blood or diet have often, but not always, been related to higher prevalence or incidence of certain age-related changes in the macula. Dietary antioxidant nutrients related to lower occurrence of AMD include vitamin E [146,151,152] or one or more carotenoids (reviewed in Ref. [153] and subsequently reported in Refs. [154–157]) or zinc [146,151,154–158]. In the Rotterdam Eye Study [146], a 35% lower risk of incident AMD was observed among the 10% of participants who were consuming antioxidant-rich diets (i.e., diets with above the median intake of all four antioxidants in AREDS supplements) compared with above median intake for only one. In this sample, high dietary intake of antioxidants particularly lowered the risk of developing AMD associated with high genetic risk [159].

Although the level of antioxidant vitamins in foods is usually lower than in high-dose antioxidant supplements, the number of different known and unknown antioxidants in foods is likely to exceed those in supplements. Foods high in antioxidants, such as vegetables, might lower oxidative stress to a greater degree than supplements. In a recent randomized, crossover trial, eating two or more cups of brassica vegetables (such as broccoli) lowered a urinary marker of oxidative stress, whereas moderate levels of supplementation with antioxidants in multivitamins did not [160]. A number of the flavonoids have been documented to have antioxidant or antiinflammatory activity or increase ocular blood flow (reviewed in Ref. [161]). The polyphenol, resveratrol, found in foods like grapes, peanuts, wine, raisins, and some berries, was found to inhibit damage to human retinal pigment epithelial cells due to substances in cigarette smoke [162]. However, the evidence regarding the effectiveness of these compounds in limiting AMD-related pathology is limited and conflicting. The impact of decades of consuming dietary nutrient and nonnutrient antioxidants on AMD may be substantial [163] and has not been adequately studied.

High-dose antioxidant supplements are considered a standard of care in the United States for individuals who have intermediate AMD or advanced stages of AMD [164]. This is based on results of AREDS, a 6.2-year randomized, placebo-controlled clinical trial, in which high doses of a combination of antioxidants (500 mg of vitamin C, 400 IU of vitamin E, 15 mg of beta-carotene, and 80 mg of zinc along with 2 mg of copper) slowed the progression of AMD from intermediate to more advanced stages by 28% [165]. The beneficial effects of the AREDS formulation persisted over 10 years, at which time there was a 27% reduction in risk of developing advanced AMD [166]. There is also evidence that this treatment reduces the age-related oxidation of cysteine in the blood, which supports the possibility that the benefit is due to a reduction in oxidative stress [167]. There was additional efficacy of the AREDS formulation when beta-carotene was replaced with L and Z (12 mg), particularly in those with low dietary intake of L and Z [168].

Genetics might modify the benefit of AREDS supplements on the rate of progression to advanced AMD, but the evidence is inconsistent. The inconsistent evidence was recently discussed by Seddon and colleagues [169] who observed a protective effect of these antioxidants and zinc only among persons with the nonrisk genotype for

CFH or *risk* genotype for *ARMS2*; whereas no modification be genotype was observed by others [170]. Whether the differential effects would be observed in other study samples or among persons taking these supplements over longer periods of time is unclear.

There is no evidence that people who have early AMD or who are at risk for AMD because of a family history will benefit from such high-dose antioxidant supplements. The levels of antioxidants that are safe and effective and efficacy of other supplement formulations over the long term is not well-studied. Results of a large meta-analysis suggest that vitamin A, beta-carotene, and vitamin E at high doses may increase mortality [171].

F Lutein and Zeaxanthin

L and its structural isomers Z and MZ are selectively concentrated in the retina where they are thought to protect against damage due to light and oxidative stress which would otherwise promote AMD (recently reviewed in Ref. [12]). They may also lower AMD risk by lowering inflammation in the retina, as a result of light damage in the retina (discussed later) or in other areas of the body [30,172] by suppressing an inflammatory response, which could indirectly promote AMD.

The highest density of these carotenoids in the central macula is found in the inner retina, in the Henle fiber and inner plexiform layers [173], where they comprise a yellow pigment referred to as MP. At these locations, they are likely to function as an optical filter that absorbs short-wavelength visible (blue) light [174]. This might protect against AMD or simply enhance vision (as discussed in Section II) They are also present in the lipid-rich POS where evidence suggests that they are likely to act as antioxidants in specific membrane domains which are rich in unsaturated lipids that are vulnerable to oxidation [175].

In the central macula, Z isomers predominate and include both Z and the L metabolite, MZ. The MP density reduces about twofold between the central macula and the periphery of the fovea, where the L isomer predominates [17]. The total concentration reduces 100-fold from the center of the cone-dominated fovea to the rod-dominated peripheral retina [176].

One mechanism by which the absorption of blue light by MP could protect against the development and progression of AMD is by blocking light-induced formation of a toxic di-retinal conjugate, A2E. Drusen deposits, characteristic of early AMD, which may be promoted by inflammatory processes [177], contain lipofuscin, the principal component of which is the toxic compound, A2E, which forms as a consequence of light-related vitamin A cycling in the retina. A2E accumulates in the RPE during phagocytosis of the rods and cones, and it is taken up by the lysosomes. A2E, in excess levels, has a variety of potential toxic effects, one of which is sensitivity to blue-light damage [178]. When a critical intracellular level of this compound has been reached, cell damage to DNA occurs, induced by blue-light irradiation [179]. Because MP could absorb 40−90% of incident blue light [174], it could reduce A2E toxicity.

There is evidence in animals that L and Z protect areas of the retina which are influenced by AMD in humans. Primates and some birds which selectively accumulate L and Z in their eyes are best to study the impact of MP on AMD or related photoreceptor health. Primates fed diets deficient in these xanthophylls [18,28] suffer a loss of RPE cells and increased photoreceptor cell death [180]. Subsequent supplementation with L protected the fovea of the retina against blue-light damage [181]. Retinal Z has also been demonstrated to prevent light-induced photoreceptor death in quail [182]. In mice, dietary L supplementation has been reported to inhibit inflammatory and angiogenic molecules related to neovascularization, similar to the inhibition of inflammatory and angiogenic processes in human retinal cell cultures [183,184].

The evidence for protection in humans comes mostly from epidemiological studies and clinical trials. In observational studies, lower rates of advanced stages of AMD have been associated with higher dietary levels of these carotenoids [185−190]. Associations between the intake or serum levels of L and Z and earlier stages of AMD are inconsistent [146,151,156,185,187,188,190]. However, results from the Carotenoids in Age-Related Eye Disease Study indicate that a protective association between dietary L and AMD is observed after excluding people who have made marked dietary changes and in women <75 years of age, in whom, the association is less likely to be influenced by survivor bias [156].

The relationships between dietary L, Z, and AMD may depend on a person's ability to accumulate carotenoids from the diet or supplements. A substantial amount of evidence (recently reviewed in Refs. [12,191]) suggests that many individual characteristics and aspects of individuals' diets modify the uptake of L and Z into the body and eye. Briefly, having indicators of metabolic stress (such as high levels of body fat, diabetes, or high levels of triglycerides) [20,192] or certain genetic variants [36,193−195] are related to lower MP density and/or the ability to increase MP with L and Z supplements [196].

A variable ability to accumulate carotenoids from supplements over the short term (2 months to 2 years) has been observed (recently reviewed in Ref. [12]). Variable accumulation in these studies might also reflect differences in supplement composition (with respect to the three xanthophyll carotenoids L, Z, and MZ) or doses, or other ingredients present in the supplement (such as omega-3 fatty acids or other antioxidants) or diets of individuals. The results of some small studies indicate

that the presence of lipids [197,198] or egg in foods [199,200] or omega-3 fats in supplements [201] might enhance bioavailability and the macular accumulation of these carotenoids.

Synergistic relationships between dietary L and genetic predisposition for AMD are possible and may explain some inconsistencies in relationships of dietary L to AMD. The presence of the common genetic risk variant in the *CFH* gene (*Y402H*), which increases risk for AMD, was less predictive of AMD in people whose diets were rich in L and Z [159,202].

The strong biological plausibility that MP protects against degeneration is supported by the observation of lower levels of L and Z in autopsy specimens of donor eyes with AMD, compared with donor eyes in people without AMD [176]. However, to date, relationships of AMD to MP density, measured noninvasively in living persons, have not been detected in cross-sectional studies [203–207]. This may be due, in part, to bias as a result of recent L supplement use in people who have been diagnosed with or have a family history for macular degeneration or survivor bias common in epidemiological studies of older people. Significant [207] or marginally significant [204] trends for a protective association have been observed when such people are excluded from analyses. A trend in the direction of protection of MP on AMD progression was observed in the only longitudinal study [208], but it was not statistically significant, nor well powered (only 27 people developed AMD over 10 years). Larger longitudinal studies that evaluate the magnitude of protection that higher MP density may have on lowering risk for AMD incidence and progression are underway, with results expected in 2018.

Even if MP density is found to be related to lower risk for developing or worsening AMD in future studies, it will remain to be determined whether this is due to dietary intake of L and Z, or the many other components in fruits and vegetables or healthy lifestyles in people with L-rich diets that may slow the development of AMD. Women who had a combination of healthy lifestyles (fruit and vegetable-rich diet, not smoking and high levels of physical activity) had higher MP density than women who did not, despite having only slightly greater intake of L and Z [141]. This suggests that many possible lifestyle factors may influence accumulation of MP and these factors may be other means of enhancing MP density.

Dietary or supplemental L, Z, and/or MZ could improve several aspects of vision in individuals with early or advanced AMD (summarized in Section 1A). However, whether this substantially improves the daily life of persons with AMD is unclear. Despite the possibility that vegetables, particularly green vegetables and dark leafy greens, may lower risk for AMD (and cataract as discussed previously), some older people limit their intake because they contain high levels of vitamin K, which could interfere with warfarin that has been prescribed to prevent blood clotting. A sudden increase in vitamin K intake from these foods can reduce the effectiveness of the drug. However, patients can consult with their health care team to have their warfarin dose titrated to the highest daily green vegetable intake that the patient can consistently eat.

G Zinc and Other Metals

Zinc may be particularly important to the retina because concentrations of zinc in the retina exceed those elsewhere in the body, with the exception of the prostate [209]. Deficiency of zinc in both animals and people impairs retinal functioning, as previously reviewed [210]. There is evidence for numerous mechanisms (catalytic, regulatory, and structural) [211] by which zinc could influence retinal integrity. Zinc catalyzes enzymatic reactions and is a cofactor of more than 100 enzymes, some of which are involved in oxidant defense. Zinc depletion in RPE cells has reduced levels of catalase, glutathione peroxidase, and metallothionein and has reduced ability to phagocytize POS [212]. Zinc performs structural roles; it facilitates protein folding to produce biologically active molecules (zinc fingers). Zinc is also involved in immune responses [213,214]. Both zinc deficiency and excesses impair immunity [214]. Zinc binds to and inhibits the activity of factor H protein in the complement system and has been proposed to contribute to deposit formation and inflammation associated with AMD [215]. Zinc depletion may also trigger apoptosis of RPE cells or increase the vulnerability of RPE cells to photic injury [216]. However, zinc supplementation can also enhance stress-induced effects in RPE cells [217]. Both lower and higher [218] levels of zinc in the choroid and RPE were observed in autopsy specimens from patients with AMD compared with those without the condition [219].

The long-term benefit of zinc on risk for AMD is suggested by the results of several but not all observational studies (reviewed in Ref. [220]). The use of high-dose zinc supplements (80 mg as zinc oxide, along with 2 mg of cupric oxide) for 6 years with or without antioxidants was associated with modestly lower progression from intermediate to advanced AMD in the AREDS [165]. Benefits persisted over 10 years of follow-up [166]. One smaller zinc supplementation trial had previously reported a benefit of zinc supplementation on vision loss in patients with AMD [221], whereas another did not [222]. In two short (6 months to 2 years) small randomized controlled studies in patients with early AMD, zinc supplementation modestly improved visual acuity, contrast sensitivity, and photostress recovery [221]. No serious safety issues

with zinc supplementation were identified in the 6-year AREDS study (aside from more frequent hospitalization for genitourinary problems in men and more frequent reports of anemia, unsupported by differences in hematocrit), and zinc supplementation was related to lower mortality in this sample [223]. This effect persisted for over 10 years of follow-up [166]. However, the long-term benefits and risks of zinc supplementation at the high levels tested in AREDS are unknown. A differential effect of zinc supplementation, based on the presence of AMD-risk alleles, has been reported in some studies [224–226], but these results were not confirmed in a larger sample of the same study population [170,224,227].

That a protective association of zinc on AMD is observed in most, but not all observational studies and clinical trials may reflect the possibility that protection is limited to individuals who are exposed to high levels of heavy metals through cigarette smoke, diet, or other environmental contaminants. Such high-risk individuals may comprise different proportions of the study samples in observational studies and trials. Two metals have been suggested to place persons at high risk for AMD, cadmium and lead [228]. These divalent cations often compete for the same binding sites as copper and zinc and have the capacity to displace these essential metals [229]. In one recent cross-sectional study in the Korean population, blood levels of lead, cadmium, and mercury were associated with a high odds for having late AMD, whereas, high blood levels of zinc and manganese (another divalent cation) were associated with lower risk.

Cadmium, a naturally occurring metal which is dispersed in the environment as a by-product of industrial activities, smoking, and fertilizers, is a potent inflammatory agent and increases oxidative stress [230]. Cadmium levels in the retinal tissues were approximately double in smokers compared to nonsmokers [231] and may explain, in part, the higher risk of AMD in smokers. Higher urinary cadmium levels were found in smokers who had AMD compared to smokers who did not have AMD [232]. Lead is also a naturally occurring metal which accumulates in our bodies with age as a function of smoking, drinking water, and other types of environmental contamination. Like cadmium, lead can contribute to the production of inflammatory cytokines and oxidative stress. Lead in the neural retina has been associated with the presence of AMD [228].

Iron, another divalent cation, is essential for retinal function, but in excess can also be toxic to the retina by catalyzing the production of reactive oxygen species, causing oxidative damage. A recent review summarizes the evidence for the beneficial and toxic effects of iron [233]. The current body of evidence suggests that iron excess, rather than deficiency, is related to AMD risk. Accumulation of iron has been observed in the retinas of persons with AMD, but it is unclear whether iron accumulation is a cause or consequence of AMD. Higher levels of serum ferritin were weakly associated with higher odds for AMD in one study in the Korean population [234]. Observations of common variants in a gene that encodes a soluble transferrin receptor are related to the odds of having AMD [235] and medical approaches to chelate excess iron are being considered as a strategy which might lower the progression of AMD [233].

In summary adequate levels of zinc from foods (dairy, meat, or beans) is associated with lower risk for AMD in observational studies, but results are limited and not consistent. Supplementation with zinc lowered the risk of progression of AMD and improved visual acuity in some studies. The benefit of zinc intake from diet or supplements on AMD risk may depend on exposure to toxic metals which compete with zinc, but has been understudied.

H B Vitamins

The importance of B vitamins to eye health has been illustrated by their ability to resolve nutritional amblyopias occurring as a result of B vitamin deficiencies and exacerbated by conditions which interfere with the absorption and metabolism of B vitamins, such as chronic alcohol overuse (reviewed in Ref. [2,236]). However, an increasing body of evidence from observational studies [237,238] and one randomized clinical trial [239] suggest that improved status of B vitamins also lowers risk for the development and progression of AMD.

There are several mechanisms by which B vitamins may play a role in AMD. The most widely considered mechanism is through prevention of hyperhomocysteinemia, which was associated with increased risk for late AMD in several past studies [237,240]; a similar, but nonsignificant, trend was observed among non-Hispanic White Americans, in a third study [241]. Folate (B9), cobalamin (B12), pyridoxine (B6), and riboflavin (B2) deficiencies either singly, or in combination, can elevate homocysteine. In two of these studies, high folate levels were associated with lower risk of one or both types of late AMD [237,238]. In one small study, low plasma vitamin B12 levels were associated with having exudative AMD, compared to patients with geographic atrophy [242]. The fortification of foods with folaic acid might lead to a substantial increase in folate status which is thought to exacerbate manifestations of vitamin B12 deficiency in blood [243] and the central nervous system [244,245,246]. Trends for the increasing intake of folic acid-fortified foods and vitamin supplements has the potential to influence AMD risk in the future.

Vitamin B6 appears to play a role in inflammation and oxidative stress; both mechanisms are known to

contribute to AMD risk. High blood levels of the active form of vitamin B6 (pyridoxal 5-phosphate) are associated with low markers of inflammation and oxidative stress [247]. Flavins, derived from riboflavin, are highly concentrated in the retina, in the space between the RPE and the rod and cone photoreceptors and are needed for photoreceptor energy metabolism and function [248]; energy metabolism is particularly high in the outer retina containing photoreceptors because of a high rate of daily renewal of the outer segments and energy is needed in phototransduction.

In summary, a benefit of supplementation of B-vitamins is suggested by evidence from one clinical trial and two observational studies. However, it is not possible to discern which B vitamin(s) were responsible and supplementation with folic acid might be harmful to some people. Additional long-term prospective cohort studies are needed.

I Vitamin D

Vitamin D is hypothesized to suppress localized inflammatory responses that occur in the retina and are implicated in the pathology of AMD [246]. The VDR is found on cells of the immune system [108,250,251] and vitamin D has been shown to alter the proliferation and differentiation of immune cells, decrease immunoglobulin production [252–256], and promote a Th1 over a Th2 cell response [108,250–252]. Evidence of the VDR on human retinal cells [107,257,258] and evidence of decreased retinal inflammation in studies of vitamin D_3 supplemented mice [259] support this hypothesis. Vitamin D is also hypothesized to prevent development of late-stage, neovascular AMD. Endothelial cells express the VDR [260] and studies in cell culture [261,262] and animal models of retinal disease [263,264] show that vitamin D has antiangiogenic properties.

The first report of a protective association between vitamin D status, assessed with 25(OH)D concentrations, and AMD was observed in a sample of participants from a nationally representative survey of the U.S. population [265]. A statistically significant protective association was found with prevalent early, but not late, AMD. Analysis of a different nationally representative survey in Korea was unable to replicate these findings with early AMD, but did observe a protective association with late AMD, but only in men [266]. Analyses in cohort studies have observed conflicting results. A protective association between serum 25(OH)D and early, but not late, AMD was observed in a cohort of postmenopausal women <75 years of age [267], but not in population-based cohorts in France [268] or the United States [269]. Further, a large cross-sectional study of members of Health Maintenance Organization found no association between medical chart-derived vitamin D status and AMD status determined from medical diagnosis codes [270]. Null results for the association between vitamin D status and nonneovascular or neovascular AMD were observed in a retrospective chart review of Medicare beneficiaries' claim files [271].

Results of case-control studies support a protective association of vitamin D on AMD. Graffe et al. observed that cases, as compared to controls, were more likely to have 25(OH)D <50 nmol/L. A total of 24 of the 31 cases of AMD had late-staged disease [272]. Itty et al. found significantly lower concentrations of serum 25(OH)D among 146 patients with neovascular AMD than in patients with either nonneovascular AMD ($n = 146$) or controls ($n = 100$) [273], but Morrison et al. reported no statistically significant differences in 25(OH)D between 50 extremely discordant AMD sibling pairs [274].

One study has reported an association between AMD and polymorphisms in the *CYP24A1* gene (which encodes the enzyme that catabolizes the active vitamin D hormone, $1,25(OH)_2D$) [274]. A different study was unable to replicate this finding [267] but did observe that associations between 25(OH)D and AMD were modified by the presence of high-risk AMD genes.

Observations of a protective effect of blood measures of vitamin D on AMD are supported by the observation that high compared to low intake of milk [265], vitamin D from foods [275,276], and vitamin D from food and supplements combined [267] have been associated with a decreased odds of AMD. One study did not find a statistically significant association with dietary vitamin D intake [277]. Interestingly, previously reported dietary associations between AMD and fish, omega-3 fatty acids (found in fish), and zinc (for which milk is an important source) (*see previous sections in this chapter*) might reflect, in part, a protective effect from dietary vitamin D.

Collectively, the existing data suggest that vitamin D might be another nutrient that protects the aging retina. Studies to date are limited by cross-sectional designs, with only Day et al. [271] investigating associations between vitamin D status and incident disease (assessed from a Medicare claims review). Many existing studies are limited because they primarily have cases of early, not late, disease. For example, the existing studies conducted in national surveys or cohorts have not had adequate cases to investigate associations with late-staged disease [265–269]. Studies of vitamin D and AMD are also difficult to assess because of potential residual confounding or overadjustment from lifestyle factors highly associated with vitamin D status [278] or from genetic risk factors for AMD. Observed associations between AMD and food and supplement sources of vitamin D should be examined with caution as total intake of vitamin D from foods and supplements may not reflect an individual's true vitamin D status [278] as vitamin D can

also be obtained from sunlight exposure. Potential confounding from the detrimental effects of sun exposure, as sun exposure is needed to endogenously synthesize vitamin D [109], adds to the complexity of interpreting epidemiologic studies of vitamin D and AMD. Sun exposure may also increase risk for AMD (reviewed in Ref. [279]). The avoidance of excessive sunlight to minimize risk for skin cancers, cataract, and AMD might jeopardize systemic vitamin D status in older people leading to potentially increased risk of AMD if adequate vitamin D status is shown to be protective. If confirmed, vitamin D status could have an impact on risk for AMD, given that portions of the U.S. population are at risk for poor vitamin D status [280,281]. Continued investigation of this potential association is warranted, especially in cohort studies powered to examine the progression of disease from early to late stages. Since AMD has such a strong genetic influence, it is also important to continue to understand the role of vitamin D status and diet in those with differing genetic risk.

J Dietary Fat

There are at least three broad mechanisms by which dietary fats might either enhance or slow AMD. First, because of the high caloric density of fats, eating high-fat foods can displace other nutrient-dense foods that may have otherwise protected against AMD. Second, eating high-fat and low nutrient density foods may contribute to high body mass, which is sometimes reported to be a risk factor for AMD [282–284]. Third, fatty acids themselves have numerous biological effects as components of biological membranes and regulators of biochemical pathways. Some dietary fats increase risk for atherosclerosis, which is related to AMD risk in some studies [131,132] and in a mouse model of atherogenesis [285]. Certain fatty acids can also have direct human physiological effects on the retina by modulating oxidative stress or by the inflammatory response, which can promote AMD pathogenesis (discussed later).

In mouse models of atherosclerosis, feeding high-fat diets resulted in the accumulation of lipid-like droplets in the retina and degenerative changes in RPE cells and Bruch's membrane [286–289]. In epidemiological studies, high dietary fat levels have been generally associated with increased risk for early and late AMD [282–284,286–294], even though these associations have not always been statistically significant. Some exceptions to this trend include prevalence or short-term incidence studies with low power to evaluate associations with either early AMD [295] or advanced AMD [290,291,293].

However, there is inconsistency across studies in the type of fat that was most related to AMD. The presence of AMD was more strongly related to high intake of saturated fats in some studies [291–294,296] and to high intake of PUFAs [297] or monounsaturated fatty acids in other studies [284,292,296]. High intake of monounsaturated fatty acids, nuts, or olive oil were associated with *lower* risk in other studies [297–299]. The intake of *trans*-fatty acids, provided in diets by margarines and other processed foods, was related to higher risk for AMD in three studies [283,284,299]. Because fat intake often changes due to alterations in food formulation and diet patterns, and particularly in relation to the common diagnosis for cardiovascular diseases which can be related to AMD, these relationships are difficult to interpret.

In addition, there is limited knowledge about the joint effects of other risk factors which might influence the associations observed. For example, chronic light exposure reduces the loss of DHA from photoreceptors in rats [300]. This suggests that light exposure may modify the impact of dietary fats on AMD. The protective association of nuts on the incidence of early AMD was greater in people without other risk factors (smoking, low dietary carotenoids and low ratio of total to HDL cholesterol in one study) [298]. However, joint effects are highly inconsistent across single studies. Larger pooled studies are needed to evaluate such joint effects in human epidemiological studies. Moreover, there is limited ability in such studies to adjust for the numerous other protective aspects of diet that accompany a more moderate, as compared to high, intake of fat.

LC omega-3 PUFAs, such as DHA or eicosapentaenoic acid (EPA), may be particularly important to the health of the retina. DHA is the most abundant LC omega-3 PUFA in rod outer segment membranes [301,302] at a concentration that exceeds levels found elsewhere in the body (reviewed in Ref. [303]). Its presence in membranes affects their biophysical properties and may influence membrane-bound enzymes, receptors, and transport. This is important in visual transduction [304], but it may also influence the pathogenesis of AMD. LC omega-3 PUFAs may protect against AMD by direct influence on retinal cell survival [305]. DHA has also been demonstrated to protect RPE cells from oxidative stress [305,306]. Deficiency of omega-3 PUFAs in nonhuman primates increases sensitivity to blue-light damage [181].

DHA might also lower risk for AMD because of its anti-inflammatory properties [307]. Numerous cell culture studies provide clues for possible mechanisms by which LC omega-3 PUFAs could enhance the integrity of vascular and basement membranes and prevent neovascularization (recently reviewed in Ref. [308]).

A mostly consistent body of evidence from observational studies indicates lower risk for early or late AMD among people with higher intake of fish, fatty fish, or LC omega-3 PUFAs [292,294–296,298,299,309,310]. Two studies found no such associations [291,297]. However, curiously, the rates of intermediate and advanced AMD

(characterized by pigmentary abnormalities and geography atrophy) in a sample of older adults from Iceland are markedly higher than rates in three other populations of European ancestry [311], despite having the highest per capita fish intake among Europeans and the fact that 56% report to use cod liver oil, a rich source of LC PUFAs.

Clinical trials have not provided strong support for the benefit of omega-3 supplements. The addition of 350 mg/day of DHA and 650 mg/day of EPA to high-dose antioxidants in the AREDS2 trial did not lower the progression to late AMD in persons who already had intermediate AMD or late AMD in one eye [312]. A lack of effect of omega-3 supplementation (840 mg/day DHA and 270 mg/day EPA) was also observed in a trial of omega-3 supplements alone [313]. A lack of effect in clinical trials or inconsistencies in protective associations in observational studies or across populations, who vary in intake of omega-3 PUFAs, could result from modifying effects of genetic risk for AMD, from the presence of genotypes which influence the synthesis or metabolism of LC omega-3 PUFAs, or the dietary content of other fatty acids. In one clinical trial, a beneficial effect of LC PUFAs was observed only in persons lacking AMD risk alleles for the *ARMS2* genotype [314]. Consistent with this modifying influence of *ARMS2* genotype, lower risk for one form of late AMD (geographic atrophy) in one observational study [315] and early AMD in a Dutch study [159] was observed in persons who had *ARMS2* risk alleles. Weekly fish intake in persons who had two *CFH* risk alleles in an Australian cohort reduced the risk for late AMD [316].

A protective influence of omega-3 PUFAs could also be dependent on the omega-6 content of the diet. A higher ratio of omega-3 to omega-6 PUFAs could increase formation of anti-inflammatory eicosanoids from omega-3 PUFAs, because the omega-6 PUFAs compete for the desaturase enzyme that creates them or replace the omega-6 PUFA content of membranes. A high ratio of omega-6 to omega-3 PUFAs also upregulates genes involved in lipid trafficking in the neuroretina [317]. In the past three studies, the risk reduction associated with a high intake of LC omega-3 PUFAs was stronger among subjects who had low intake of omega-6 PUFAs [283,284,296].

K Herbal Supplements

The use of herbal supplements has increased in the United States. Several herbal supplements, such as those containing ginkgo biloba and bilberry, have been promoted to benefit the health of the retina. However, there are no scientific studies that support their benefit except one very small (20 persons) study of ginkgo biloba in patients with AMD, in which improvement in visual acuity was indicated in a preliminary report (previously reviewed in Ref. [318]).

V DIABETIC RETINOPATHY

A Overview

DR is a complication of diabetes that is considered to be the result of damage to the microvasculature of the retina. It is the leading cause of new cases of blindness in working aged U.S. adults (20–74 years) [319]. Approximately 12.3% of Americans aged 20 years and older have diabetes, and this is estimated to increase to 14.0% by 2030 [320]. Diabetes is especially burdensome in minority populations, such as African Americans, Mexican Americans [321], and Native Americans [322], in whom the prevalence and incidence are higher than the national average. The burden of associated complications like DR will likely also increase in the coming years.

Previous work has described the natural history of the disease (reviewed in Refs. [319,324]). In brief, preclinical stages of DR include changes in retinal blood flow. Nonproliferative diabetic retinopathy (NPDR) consists of the formation of clinical lesions (microaneurysms and intraretinal hemorrhages), the appearance of retinal exudates (lipid deposits from leaky blood vessels) and cotton wool spots (resulting from localized ischemia), and the appearance of venous bleeding and loops. Blindness can result at these stages if macular edema occurs. In the later proliferative stage, proliferative diabetic retinopathy (PDR), new vessels and fibrous tissue can originate from the optic disc or elsewhere in the retina. Problems arise if the vessels grow through the inner limiting membrane into the vitreous humor of the eye, which is constantly contracting and condensing. Often this movement can lead to vessel tear, hemorrhage, and blindness.

Limited treatment options exist that include invasive injections of antiangiogenic agents into the vitreous for PDR [325] and laser photocoagulation [323], to cauterize leaking, newly developed blood vessels. Both injections of antiangiogenic agents and laser photocoagulation involve investing time in treatment and may cause discomfort. Photocoagulation does not necessarily prevent vision loss in all individuals, and can result in peripheral vision loss or decreased dark adaptation [326].

B Causes

Randomized controlled trials have demonstrated that maintenance of tight blood glucose control via intensive insulin therapy is associated with lower incidence and progression of DR in individuals with type 1 and 2

diabetes [327−330]. Even so, intensive insulin therapy did not prevent the occurrence of DR in all individuals and was often associated with increased bouts of hypoglycemia [327] Other well-established risk factors for DR are duration of diabetes [324], hypertension [332], and elevated blood lipids [333].

Various types of diets, foods, or nutrients may influence risk for DR either by affecting (1) blood glucose control, (2) blood pressure, (3) serum lipid levels, or (3) via other mechanisms (e.g., those related to the antioxidant or antiinflammatory activity of differing diets, foods, and nutrients) (summarized in Table 19.3). Nutrition therapy for patients with diabetes could be targeted at preventing DR or its progression and may be less costly than current treatment. This section will highlight areas believed to be the most robust with respect to research on nutrition and DR.

C Healthy Diet Patterns

An accumulating body of work supports a protective association between DR and consumption of a healthy diet, one high in fiber and low in saturated fat (and most likely rich in certain micronutrients). To date, most work on diet patterns has involved examining the association between DR and numerous foods and nutrients individually rather than examining index-derived dietary patterns in relation to DR, as done in other studies of diet and chronic disease [53,141,334,328]. Previous studies have observed protective associations between DR and intake of fruits and vegetables [329−339]. Fruit and vegetable-rich diet patterns, like the Dietary Approaches to Stopping Hypertension diet, might lower DR risk by lowering blood pressure or other risk factors for DR [340]. Roy et al., in a study of African American participants [341], observed an increased risk of progression to macular edema with high, compared to low, sodium intake. However, two recently published, robust analyses have not corroborated these findings [329,342]. These studies considered sodium intake alone, not as a component of a more complex dietary pattern. Recently, findings from a post hoc analysis of a randomized dietary modification trial in individuals with type 2 diabetes observed a protective effect of a Mediterranean Diet (MedDiet) plus extra virgin olive oil, as compared to a low-fat dietary pattern, on the risk of sight-threatening DR over 6 years of follow-up [343]. A MedDiet likely improves DR by reducing blood pressure and improving blood glucose control and lipid profiles; as well as through other mechanisms involving its influence on oxidative stress, inflammation, and promotion of a healthy endothelial function (reviewed in Refs. [344,345]). More work on associations between dietary patterns would be beneficial in understanding the complex role of diet in DR incidence and progression.

D Alcohol Consumption

Research has shown a protective association between moderate alcohol consumption and risk of coronary heart disease and mortality in individuals with type 2 diabetes [346]. These finding have led to recent interest in investigating the association between alcohol consumption and diabetic microvascular disease, such as DR [347,348]. Moderate alcohol consumption is hypothesized to be protective due to a number of different mechanisms related to cardiovascular health and DR risk factors (e.g., inflammation, dyslipidemia and insulin resistance, platelet aggregation), reviewed in more detail elsewhere [349,350].

Alcohol consumption in relation to DR has been studied in a number of observational studies, many of which have found no association between alcohol consumption and DR [351−357]. In 1984, Young et al. reported that heavy alcohol consumption was associated with the development of DR, primarily exudative and PDR, in a sample of 296 men [358]. Since then, a handful of studies have observed protective associations between alcohol consumption and DR, primarily moderate consumption. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy, average alcohol consumption was associated with the decreased odds of prevalent PDR among individuals with diabetes onset before age 30 [359]. Analyses examining alcohol's association with the incidence and progression of DR in the same sample, albeit a smaller subset, did not observe the same association [355]. Beulens et al. observed that moderate alcohol consumption was associated with the reduced odds of prevalent PDR among 1857 participants with type 1 diabetes [360]. Two recently published cross-sectional studies [347,361] support a protective association with moderate [347] or light [361] alcohol consumption and reduced odds of any DR or severe DR, respectively. These studies [347,361] also suggest associations may vary by beverage type. Further, a 5-year prospective study observed a protective association of moderate alcohol consumption on the incidence of microvascular disease among 11,140 individuals with type 2 diabetes [348].

Many of these studies are limited by their cross-sectional designs as well as limited data on alcohol consumption quantity and patterns. Further, if alcohol consumption is mostly influential on the development of PDR, smaller studies limited in the number of advanced cases of disease might not have the power to observe an association if it existed. The findings to date are intriguing and deserve further study. Continued work in the area of dietary

TABLE 19.3 Summary of Evidence Relating Diet to DR

Nutritional Exposure	Strength of Evidence	Comment
Healthy Diet Patterns	*A diet rich in fruits and vegetables, micronutrients, and high in fiber is likely beneficial.* Observational studies support a protective association between high fruit and vegetable consumption and high fiber intake with the reduced odds or risk of DR. A post hoc analysis of a dietary modification trial comparing those randomized to a MedDiet with extra virgin olive oil to a low-fat dietary pattern showed reduced risk of incident sight-threatening DR. Such a diet may be beneficial by helping maintain blood glucose control and by lowering blood lipids and hypertension.	Diets rich in fruits and vegetables are likely high in fiber and rich in other micronutrients.
Alcohol Consumption	*Benefit of light to moderate alcohol consumption on reduced risk of DR is suggested.* Protective associations between moderate alcohol consumption and coronary heart disease led to research on the role of alcohol and DR. Four cross-sectional studies and one prospective study support a protective association between light to moderate alcohol consumption on risk of DR or microvascular disease. Three of these studies show an association with severe or PDR.	Light to moderate alcohol consumption could reflect an overall dietary pattern or lifestyle that protects against DR. The role of alcohol consumption as a risk factor for DR should be continued to be studied, but with consideration of alcohol as part of an overall dietary pattern. Many previous studies are limited by the lack of detail collected on alcohol intake, including the quantity of alcohol consumed, the frequency of consumption, beverage type usually consumed, and duration of consumption.
Dietary Fat and Fiber	*Diets low in saturated fat but high in omega-3 fatty PUFAs are likely beneficial.* A few epidemiologic studies show associations between high saturated fat intake and an increased odds of DR, although results are not consistent. A recent large prospective analysis showed a decreased risk of sight-threatening incident DR among participants consuming >500 mg/day of omega-3 fatty acids. *Benefit of high fiber diets are likely:* There is consistent evidence from observational studies supporting a protective role of dietary fiber intake and high fiber food intake (with an emphasis on soluble fiber) and reduced risk of DR. High fiber intake may be beneficial in helping maintain blood glucose control.	To our knowledge, only one study has specifically studied the influence of LC omega-3 PUFA intake on DR. More research is needed.
Antioxidants	*Benefit of foods rich in antioxidants is likely but inadequately studied.* Studies from diabetes-induced animal models strongly support a protective effect of antioxidant intake and maintenance of a healthy retina. Findings from both small patient and population-based samples are inconclusive. Findings from larger epidemiologic studies examining associations between dietary intake or serum concentrations of antioxidants do not conclusively support a protective effect of antioxidants on DR. Some studies suggest that antioxidant exposure could be potentially detrimental; however some of these findings may be explained by bias inherent in cross-sectional study designs. One clinical trial, in a post hoc analysis, found no association between vitamin E supplementation and history of DR laser therapy.	Observational studies are limited in number and mostly are cross-sectional in design. Results may be biased by changes in diet and supplementation practices upon development of diabetic complications. Long-term observational prospective studies are needed.

(*Continued*)

TABLE 19.3 (Continued)

Nutritional Exposure	Strength of Evidence	Comment
	One observational study suggested that long-term intake of antioxidant supplements or multivitamins may protect against DR.	
Vitamin D	*Benefit of good vitamin D status (from adequate sunlight, foods, and/or supplements) is possible.* In the last 5 years, research on the possible protective association of vitamin D status with DR has led to over a dozen published studies on this topic. Many are limited by their cross-sectional designs. Data from prospective studies are not consistently supportive of a protective association.	Continued examination of this association in prospective studies is needed. Studies should be powered to examine progression of DR, especially progression to PDR. Studies able to adjust for pertinent confounding variables are needed as higher blood levels of vitamin D could be related to other aspects of diet or lifestyle that could protect against DR.

patterns may provide insight into the influence of alcohol, as a part of an overall dietary pattern, on DR.

E Dietary Fat and Fiber

Previous studies have also observed that high calorie and fat (% kcals), both total and saturated, intake may increase risk for DR. A small case-control study in India observed that diets high in energy, animal proteins, and animal fats were more common among persons with PDR than among controls [339]. Data from one ecological study showed a greater prevalence of DR in persons with type 1 diabetes in European regions with higher, compared to lower, mean intakes of cholesterol, total fat, and saturated fat (% kcals) [362]. Further, the Diabetes Control and Complications Trial (DCCT) reported that conventionally treated participants who followed the American Diabetes Association dietary recommendations with respect to fat and total calorie intake were less likely to experience DR progression [363]. Sasaki et al. also observed an increased odds of DR among those consuming high compared to low intake of energy-adjusted, saturated fatty acids, but only in those with good glycemic control; no associations were found for total or monounsaturated fatty acid intake [364]. A prospective study in 649 African Americans with type 1 diabetes observed that total kilocalories along with age, glycosylated hemoglobin (Ghb), and hypertension were found to be significant predictors of vision-threatening DR and severe hard exudates [341]. The authors suggest that increasing kilocalories increases hyperglycemia and dyslipidemia and thus oxidative stress, a proposed etiologic mechanism for DR [365]. Differently, two cross-sectional studies [366,367] observed no statistically significant differences in total calories, total fat (% kcals) [366,367], or saturated fat (% kcals) [367] consumption between persons with and without prevalent DR.

Some of the original investigations of the associations between dietary intake and DR were conducted in the 1980s and involved small intervention studies of linoleic acid, an omega-6 PUFA, among individuals with type 2 diabetes [368,369]. These studies suggested that intake of diets enriched with linoleic acid decreased development and progression of DR, especially among those with poor glycemic control. The authors hypothesized that linoleic acid enrichment of cell membranes would increase the sensitivity of the insulin receptor and thus improve blood glucose control [368]. However, these earlier intervention studies' effects could have been due to linoleic acid consumption displacing consumption of other more harmful fatty acids, such as saturated fat. A recent finding in a sample of 379 patients with diabetes supports a protective association with DR among those consuming high compared to low intake of PUFAs but only in those with good glycemic control [364]. This study did not differentiate between omega-3 and omega-6 PUFAs. A recent prospective analysis of 3482 participants with type 2 diabetes observed a 48% decreased risk of sight-threatening DR with self-reported consumption of 500 mg/day or more of LC omega-3 PUFAs [370]. The observed protectiveness of omega-3 intake on DR was hypothesized to be due to the antiinflammatory properties [371] of omega-3 oxylipins or via their proposed antiangiogenic properties [372] as shown by research in animal studies.

Diets rich in fiber, especially soluble fiber, have been shown to improve glycemic control and insulin sensitivity [373], and thus likely reduce risk for DR. This hypothesis is supported by previous findings of protective associations between consumption of high-fiber containing foods (e.g., fruits and vegetables) and DR, as previously noted [337–339,374]. The DCCT observed that intake of dietary

fiber (% kcals) was inversely correlated with progression of DR [363]. One of two studies [366,367] observed lower intake of fiber in persons with compared to without DR. More recently, a population-based, cross-sectional study of 1261 individuals with type 2 diabetes [375] observed a 41% and 24% increased odds, respectively, of overall and sight-threatening DR among persons with low compared to high consumption of fiber-rich foods with adjustment for confounding factors. However, total quantity or type of dietary fiber was not determined.

In summary, more research is needed to assess the effects of long-term calorie, dietary fat, and fiber intake with respect to incidence and progression of DR. The published studies of calories, dietary fat, and fiber have a number of limitations including the use of ecologic, case-control, or cross-sectional designs, dietary assessment methods, which do not adequately assess long-term dietary intake, lack of adjustment for confounding factors, and small sample sizes.

F Antioxidants

Hyperglycemia is thought to increase oxidative stress through a number of proposed mechanisms which include damage to DNA, lipids, proteins, and carbohydrates as well as functional alterations of a number of other metabolic pathways that promote oxidative stress (previously reviewed in detail elsewhere [376]). Increased oxidative stress is hypothesized to promote diabetic vascular complications such as DR [365,376]. The retina is especially susceptible to damage from reactive oxygen species due to the PUFA-rich endothelial cells of the retinal microvasculature [365,377].

Further, some studies have shown that individuals with diabetes compared to individuals without diabetes have lower blood levels of antioxidants [378,379], as well as lower carotenoids in the retina [20], perhaps as a result of increased oxidative stress. Antioxidant intake has been proposed to help alleviate the observed increased state of oxidative stress in individuals with diabetes and help prevent development of microvascular complications such as DR [380]. Supplementation of streptozotocin-induced diabetic rats for 12 months with antioxidant micronutrients resulted in less degeneration of the retinal microvasculature (indicating early signs of DR) [381] and better retinal function, mitochondrial homeostasis and less elevation in inflammatory biomarkers within the retina [382] than in nonsupplemented animals. However, numerous studies investigating associations between antioxidant micronutrients (primarily vitamins C and E) and DR in small patient or population-based samples have yielded conflicting results [383–385].

To date, insufficient data from robust epidemiologic studies exist to conclude that diets or supplements high in antioxidants prevent or slow DR. In a population-based cohort study no statistically significant associations were observed between dietary intake of vitamin C or vitamin E (assessed 6 years earlier) and prevalent DR [386,387]. In a population-based survey, serum vitamin C concentrations were unrelated to prevalent DR, but serum vitamin E concentrations were positively associated with increased odds of DR [388]. This relationship was attenuated when current supplement users were removed from the analysis. Mayer-Davis et al., in a different cross-sectional study, observed that the severity of prevalent DR was higher in subjects who had high intakes of vitamin C, in insulin users who had high intakes of beta-carotene, and in noninsulin users who had high intakes of vitamin E [389]. A recently published prospective study did observe protective associations between incident DR and vitamin C and carotenoid intake, but not with vitamin E [374]. Data from a clinical trial of vitamin E supplementation also did not observe differences in history of DR laser therapy between supplementation and placebo arms [390]; however, DR was not the primary endpoint for the trial. Associations of prevalent DR to short-term diet and blood nutrient levels in cross-sectional studies may be biased by the recent use of popular antioxidant supplements, particularly in people who may be experiencing more severe symptoms or complications of diabetes.

Most previous studies of antioxidants and DR have not been able to capture the measure of long-term exposure to these nutrients. In one study, participants who reported using multivitamins, vitamin C, or vitamin E supplements for 3 or more years before DR were assessed to have lower odds of DR [386]. It is possible that long-term use of antioxidant supplements may protect against DR. Only one smaller ($n = 97$) randomized clinical trial of patients with type 2 diabetes has reported the effect of antioxidant therapy with the primary outcome of DR [391]; however, the study failed to compare the change in DR stage over time between trial arms. Moreover, there exists only one [374] prospective study reporting data on the influence of dietary antioxidants on the onset or worsening of DR. Given the strong evidence for protection by antioxidants in animal studies, and the suggestion of benefit from long-term antioxidant use in one large observational study, additional prospectively designed studies are needed to better assess these relationships.

G Vitamin D

Over the last 5 years, there has been a rapid increase in the number of papers studying the association between vitamin D status and DR. For example, vitamin D has been shown to reduce the damage in cultured endothelial cells from

advanced glycation end products [392] formed in states of hyperglycemia and thought to propagate oxidative stress and inflammation. [393] The etiology of DR is hypothesized to involve hyperglycemia-induced chronic low grade inflammation [394] and advanced DR involves angiogenesis.

The majority of the work has been in cross-sectional [395–407] and case-control [408–412] studies with around half supporting a protective effect of vitamin D on DR [398,400,401,394,405,407,408,410,411]. Studies, other than those conducted in nationally representative surveys [398,392] and population-based cohorts [397,401], recruited participants from clinic settings. The degree to which this affects the generalizability of study findings is unknown. Other study limitations include small sample sizes ($n \leq 300$ for samples of individuals with diabetes) [395,399,403,394,404,407–410] and assessment of DR status from ophthalmologist examination rather than from standardized grading of retinal fundus photographs [395,396,393,403,405–412]. Only three studies to date have examined prospective associations between vitamin D status and risk of DR [413–415] with only one observing a protective association [414]. However, further adjustment of their multivariable model for Ghb, physical activity, or seasonal removed the statistical significance of the observed association. Studies of associations between polymorphisms in the VDR and DR have been inconclusive with some supporting [416–418] and others not supporting [419,420] possible associations.

Additional work in prospective epidemiologic studies are needed that can account for pertinent confounding variables as well as provide adequate cases to investigate associations between vitamin D status and PDR. Continued work in minority populations with the greatest burden of diabetes and the greatest risk for vitamin D deficiency (e.g., African Americans) [280] should be conducted.

H Summary

The last 20 years has provided a significant body of data suggesting that dietary intake and vitamin D status may be risk factors for DR with mechanisms affecting disease etiology that could extend beyond diet's influence on blood glucose control. Evidence from a large dietary modification trial has provided new evidence that fruit and vegetable-rich dietary patterns such as the MedDiet may protect against DR. Associations between DR and alcohol consumption, macronutrient intake, sodium intake, antioxidants, and vitamin D status still remain inconclusive. Prospectively designed studies are needed to evaluate the importance of these dietary components, as well as other healthy diet patterns, on the incidence and progression of DR in the general population. Given the broad aspects of diet that could protect against DR such data will particularly assist in making public health recommendations.

VI GLAUCOMA

A Introduction

Glaucoma is the leading cause of *irreversible* blindness worldwide, and is predicted to affect nearly 80 million individuals by 2020 [421]. Glaucoma is an umbrella term for a group of diseases that damage the optic nerve, the bundle of nerve fibers that connect the retina to the brain, causing loss of vision starting in the peripheral field of view. The most common form of glaucoma is primary open-angle glaucoma (POAG). In POAG, the "angle" where the cornea and iris meet is restricted. As a result, the flow of ocular fluid to nourish nearby tissues passes too slowly through the trabecular meshwork, increasing eye pressure and leading to progressive deterioration of the retinal ganglion cells that form the optic nerve. The impairment of vascular supply to the optic nerve head is an additional pathological process that is thought to contribute to the development of POAG [422].

Clinically, the focus of glaucoma treatment has been on management of intraocular pressure (IOP). Elevated IOP (reflecting either excessive aqueous humor production or inhibition of aqueous humor outflow) contributes to ganglion cell damage and progressive loss of vision, starting in the periphery of the field of vision. IOP can be modified surgically or through the application of topical medications [423]. However, glaucoma may develop in those who have normal IOP [424], suggesting that other factors may contribute to its pathology.

Age and family history of glaucoma increase risk for POAG. Globally, this condition is about two to three times more common among people of African than of European, Hispanic, or Asian ancestry. Risk has been higher in people who are obese and/or have diabetes and hypertension [436,437]. Diet could influence the occurrence of glaucoma, indirectly, by its influence on these chronic conditions. Diet can also influence IOP. The role of nutrition in both IOP and glaucoma development is still an emerging field. The current state of evidence linking diet directly and indirectly to glaucoma is summarized later.

B Obesity

A body of evidence indicates that a higher body mass index (BMI) is linked to higher IOP, the principle modifiable risk factor for glaucoma [427–430]. Change in BMI over 5 years was found to be associated with change in IOP during that time [431]. Increases in BMI, body fat percentage, and waist circumference were

prospectively linked to increases in IOP in a large cohort of Korean adults. A number of mechanisms have been put forth to explain the relationship between obesity and IOP, including high blood pressure, elevated blood glucose, increased blood viscosity, insulin resistance (and diabetes or metabolic syndrome), and oxidative stress. Hyperglycemia, which is common in individuals with high BMI, might reduce outflow of aqueous humor through the trabecular meshwork via glycosylation of extracellular matrix proteins [432] or by increasing oxidative stress (discussed later). Weight loss through diet and exercise may serve to lower IOP in glaucoma patients who are obese and/or have diabetes, but this hypothesis has not been tested in clinical trials. While there is convincing evidence that high BMI and diabetes mellitus are related to higher IOP, BMI has not been consistently related to the incidence of glaucoma [433].

C Antioxidants

A role for oxidative stress in the pathophysiology of glaucoma has emerged in recent years [434]. It has been suggested that chronic oxidative stress may contribute to death of retinal ganglion cells and to remodeling of the trabecular meshwork [435,436], an effect that may prevent proper draining of aqueous humor and increase IOP. High levels of reactive oxygen species can also contribute to signaling pathways that lead to cellular apoptosis. Therefore, dietary antioxidants could alter glaucoma risk. This includes sufficient levels of vitamins and minerals (iron, B vitamins, zinc, and selenium) that are cofactors for antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase), glutathione, and food components that are direct antioxidants (vitamins C and E, carotenoids, and numerous other plant components).

Cross-sectional observational studies support a relationship between the status of antioxidants in the blood and the presence of glaucoma [426–439]. Likewise, similar studies have demonstrated that glaucoma patients have lower total antioxidant status of the aqueous humor [439–441], suggesting that a state of oxidative stress is present in the eye as well. Although total antioxidant status may be compromised, numerous studies have demonstrated that levels of antioxidant enzymes (e.g., glutathione peroxidase, superoxide dismutase) are *higher* in the aqueous humor of glaucoma patients [442,443], suggesting that the expression of these enzymes is upregulated in response to oxidative stress. Moreover, markers of oxidative DNA damage have been found to be elevated in the trabecular meshwork of glaucoma patients [444], suggesting that the tissue responsible for aqueous humor drainage is affected by oxidative stress. The trabecular meshwork may be particularly susceptible to oxidative stress, as it has poorly developed antioxidant defense systems relative to other ocular tissues [445].

Despite the evidence demonstrating a connection between oxidative stress and risk of glaucoma, little evidence from prospective cohort studies exist to support the idea that consuming dietary antioxidants can reduce glaucoma risk or progression. Although fruit and vegetable-rich diet patterns [338] and consuming a high number of fruits and vegetables [446] were associated with a lower likelihood of having glaucoma in cross-sectional studies, large prospective studies have observed no relationship between antioxidant-nutrient consumption and risk of developing glaucoma [447]. In addition, consumption of supplements containing vitamin A or vitamin E was not associated with glaucoma risk, although consumption of vitamin C supplements was associated with mildly decreased risk in the NHANES [448].

The inconsistency in relationships between the intake of fruit and vegetable-rich diets might reflect the possibility that only some fruits and vegetables lower risk more than others. This is suggested by a recent study in which total dietary nitrate intake (high in leafy greens and to lesser extents in certain fruits and vegetables) was associated with lower POAG risk (particularly with early visual field loss in the paracentral field of vision for which dysregulation of the ocular vascular system has been implicated) [449]. This finding contributes to a substantial body of evidence that suggests a key role of the nitrous oxide system in POAG pathogenesis, which may both elevate IOP and dysregulate ocular blood flow [450].

Leafy greens that are rich in L and Z might be protective against the development of glaucoma, as with other forms of age-related eye diseases. These carotenoids are known to have antioxidant effects (See Section II). Results of two small studies suggest that MP is lower in people with glaucoma. In one case-control study the density of L and Z in macular pigment (MP) were lower in glaucoma cases than controls [451] In another study, MP levels were lower in glaucoma patients who had a loss of the foveal ganglion cell complex, compared to glaucoma patients in whom this complex was intact [452]. MP level also correlated with retinal nerve fiber layer thickness and cup to disc ratio, both considered important indicators of glaucomatous optic neuropathy. However, higher dietary levels of L and Z have not been related to the prevalence or incidence or progression of glaucoma in the few studies which have examined these associations [446,447]. Variable abilities to absorb carotenoids and transport them into the eye (Section II) might explain the inconsistencies in these findings. Additional prospective studies of levels of L and Z in the diet or MP to the incidence and progression of glaucoma are needed.

There is generally a lack of randomized clinical trials that have directly examined the effect of antioxidants on

glaucoma incidence and progression. One 2-year supplementation trial in patients with preexisting glaucoma observed no effect of antioxidants (containing L and Z) with or without omega-3 fatty acids on visual field measures or ganglion cell thickness [453]. This may be too short of a period of time to observe a protective effect if there is one.

Ginkgo biloba extract, derived from a plant commonly used in traditional Chinese medicine, has received attention as a potent antioxidant that may be useful in the treatment of glaucoma [454]. Ginkgo biloba extract improved measures of visual field in patients with preexisting normal-tension glaucoma [455,456] and slowed the loss of the visual field in this subgroup of glaucoma patients [457]. These apparent benefits of ginkgo biloba for glaucoma patients may be related to its role as an antioxidant, or to improvements in the microcirculation feeding the retina, or to the inhibition of apoptosis in retinal ganglion cells [454]. Ginkgo biloba has gained some mainstream acceptance as a useful treatment for glaucoma, but only in normal-tension glaucoma patients, or in patients whose glaucoma has progressed despite successful lowering of IOP [458].

In summary, although there is considerable evidence to suggest a link between oxidative stress and glaucoma risk, there is less evidence to demonstrate that specific antioxidants can reduce this risk. There is also evidence that other components of certain fruit and vegetables (such as nitrates, or those rich in L and Z) might lower risk by mechanisms other than lowering oxidative stress, or in addition to it. At the current stage of knowledge, it is advisable that glaucoma patients, or those at high risk of glaucoma, get their antioxidants through a healthy diet rich in fruits and vegetables, as there is little evidence that high-dose antioxidant supplementation is either effective or safe in the long term.

D Omega-3 Fatty Acids

Animal research suggests that supplementation with omega-3 fatty acids may reduce IOP by increasing aqueous outflow [459], an effect expected to slow progression of optic nerve degeneration. Furthermore, omega-3 deficiency increased ganglion cell dysfunction when IOP is elevated [460]. Consistent with the idea that LC omega-3 PUFAs might play a role in the etiology of glaucoma, POAG patients were observed to have lower levels of EPA and DHA in several fatty acid fractions derived from red blood cells [461]. The authors speculate that omega-3 PUFAs may reduce risk of POAG by improving microcirculation around the optic disc, consistent with the role of n-3 fatty acids as inhibitors of platelet aggregation.

However, results of investigations in two large cohorts, followed prospectively do *not* support a protective role of omega-3 fats and POAG. In the Nurse's Health Study and the Health Professionals Follow-Up Study cohorts [462], and in the SUN cohort [463], a higher ratio of dietary omega-3 to omega-6 fatty acids were related to *higher* risk for POAG. One possible explanation suggested is that higher omega-6 consumption leads to greater availability of prostaglandin F_{2a}, an omega-6 derivative that is believed to reduce IOP [462]. Given the larger body of evidence suggesting that a higher dietary omega-3 intake may be beneficial for cardiovascular health and lowering AMD risk, lowering omega-3 fat intake is not recommended for the general population. Furthermore, other lines of evidence suggest a protective role for omega-3 fatty acids.

E Alcohol

Acute consumption of alcohol can transiently reduce IOP [464,465], raising the possibility that consistent, moderate consumption of alcohol over time would reduce the risk for glaucoma. It has been suggested that alcohol may reduce aqueous humor formation by the ciliary body, primarily through the inhibition of antidiuretic hormone [466]. In observational studies, which reflect the impact of alcohol over a longer term, relationships of alcohol to IOP are conflicting. Alcohol use was associated with lower IOP in one cross-sectional study [467], but with *higher* IOP in another [468]. Most often, alcohol intake has not been related to IOP [356,429,469–471]. In one large prospective study, there was a trend toward lower IOP in individuals consuming >30 g alcohol (approximately two drinks) per day [470]. The inconsistent relationships of alcohol to glaucoma risk might be explained by limited statistical power and variable adjustment for other lifestyle factors associated with alcohol. Furthermore, the range of habitual alcohol intakes is fairly narrow, making it difficult to study the effect of chronic binge drinking.

F Caffeine

Caffeine is a central nervous stimulant commonly consumed by most age groups and is found abundantly in coffee, dark colas, energy drinks, and certain teas. Caffeine has diverse effects throughout the body, raising the distinct possibility that caffeine influences eye health. Data from clinical trials suggest that consumption of caffeine can raise IOP [472,473,474], but other trials have not shown any effect [475,476]. A meta-analysis of randomized clinical trials revealed that acute consumption of caffeine can transiently increase IOP, but only in patients with preexisting glaucoma or ocular hypertension [477].

The increased IOP with caffeine consumption may be attributable to increased aqueous humor production or

inhibition of aqueous humor drainage. Caffeine raised IOP in dogs by increasing aqueous humor production without affecting drainage [478]. The same group demonstrated that caffeine- induced structural changes in the ciliary epithelium that would support transportation of aqueous humor [479]. Acute caffeine consumption increases systemic blood pressure [474] and this may also contribute to aqueous humor production via increased filtration through the ciliary body.

Results from the Blue Mountains Eye Study corroborate this finding, as regular coffee drinking was cross-sectionally linked to higher IOP in glaucoma patients only [480]. These findings from cross-sectional research and randomized clinical trials support the recommendation that glaucoma patients limit caffeine consumption. Although research has not yet linked caffeine to visual field loss in glaucoma patients, it is plausible that higher IOP with caffeine use would contribute to the onset of blindness.

While there may be a connection between caffeine intake and IOP in patients with pre-existing glaucoma, little data suggests that regular caffeine consumption increases risk for glaucoma. Data from the Nurses' Health Study and the Health Professionals Follow-up Study revealed that caffeine consumption was not prospectively linked to higher rates of POAG [481]. However, a significant relationship was found for those with a family history of glaucoma, suggesting that the effect of caffeine on glaucoma risk has a genetic component. It is plausible that the transient effect of caffeine on IOP is not sufficient to damage the retina, or that the IOP response is attenuated when caffeine is consumed habitually. More longitudinal research is needed to firmly establish the relationship between caffeine dose, IOP, and glaucoma risk, and to discern whether this relationship varies according to glaucoma diagnosis.

G Summary

There is considerable emerging evidence to suggest a link between pathological processes of glaucoma (oxidative stress, elevation of IOP) and diet components. Diet might also lower risk for the development of obesity and chronic diseases that are more common in people with glaucoma. The current body of scientific evidence on relationships of diet to glaucoma suggests that fruit and vegetable-rich diets may lower glaucoma risk through either or both means. However, the data are limited and insufficient to support the possibility that the intake of specific foods or dietary supplements is likely to lower glaucoma risk. At the current stage of knowledge, it is advisable that glaucoma patients, or those at high risk of glaucoma, eat healthy diets rich in fruits and vegetables.

VII CHAPTER SUMMARY

Scientific evidence suggests that *nutrition matters* in maintaining eye health, as with other parts of the body. That is, there is mounting evidence for substances in food which exert effects that are likely to promote or delay the development of the most common and costly causes of vision loss: age-related cataract and macular degeneration and DR. An emerging, but limited, body of evidence suggests that several specific aspects of healthy diets and lifestyles might lower glaucoma risk, as well. It is possible that healthy diets and lifestyles matter more (or less) in people genetically prone to these processes. Healthy diets, other healthy lifestyles, and genetic risk might work synergistically to lower risk for age-related eye diseases. However, the evidence is insufficient to make separate recommendations for individuals based on genetic risk.

Despite the evidence that *nutrition* matters, evidence that supplements of single or combination of nutrients slow age-related cataract, macular degeneration, glaucoma or DR is more limited. An exception is for the intake of high-dose antioxidant supplements and zinc in people with AMD, which is considered a standard of care for slowing the progression of AMD. There is no strong evidence that these or other supplements *prevent* this disease, or other age-related eye diseases. In most cases, nutrient-dense foods provide a larger array of potentially protective substances than pills. Increasing evidence suggests a larger impact of foods than supplements. An exception may be for vitamin D, which is sometimes hard to obtain from natural food sources. Vitamin D status might be most important in extreme northern and southern hemispheres which provide inadequate UV light in winter to permit the synthesis of this vitamin in skin.

Optimal ocular health may begin with gestation and infancy and continue into old age. Recent evidence has emerged to suggest a possible role of L and Z in gestation and infancy; a role in lifelong health has been proposed, but not studied. The most sustainable means of promoting eye health through nutritional means in populations over the long term may be to foster the ability to breast feed and assure access to a variety of foods, a large proportion of which are plant foods.

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Chapter 20

Phytochemicals in the Prevention and Treatment of Obesity and Its Related Cancers

Kee-Hong Kim[1] and Ki Won Lee[2]

[1]*Purdue University, West Lafayette, IN, United States,* [2]*Seoul National University, Seoul, Republic of Korea*

I INTRODUCTION

The prevalence of obesity is a significant global health problem. Obesity is the result of excess body fat due to an imbalance between energy intake and energy expenditure. Energy balance is influenced by numerous environmental factors (e.g., metabolic rate, exercise, and culture), and genetic factors (e.g., monogenic syndromes and susceptibility genes) (Fig. 20.1). Indeed, the excessive body fat accumulation in adipose tissue is a consequence of impaired energy expenditure, such as reduced physical activity, basal metabolism, and thermogenesis [1]. On the other hand, an increase in intake of foods with added sugars, less fiber, and/or elevated fat content impacts positive energy balance, resulting in an expansion of adipocyte size and weight, and an increase in number of new adipocytes. In addition, complex interactions among these variables contribute not only to individual differences in adipose mass gain, but also in the response to interventions and/or treatments of obesity. Brain and peripheral tissues such as adipose tissue, liver, and intestine play an important role in regulating systemic energy balance and, therefore the development and progression of obesity. Among these tissues, adipose tissue is known to play both causative and consequential roles in obesity. Although adipose tissue is traditionally known as an energy reservoir, storing lipids during periods of energy excess and positive energy balance, generation of new fat cells, and metabolic and endocrine changes in adipose tissue also contribute to metabolic dysfunction of other peripheral tissues and dysregulation of central signals of energy balance. This suggests that the generation of new adipose tissue and its metabolic and endocrine function could be preventive and/or therapeutic targets of obesity.

Despite many reported beneficial effects of dietary phytochemicals on health, less attention has been placed on the modulation of obesity and its associated diseases by phytochemicals. Thus, this chapter focuses largely on understanding the cellular processes involved in the generation and function of adipose tissue and obesity-related cancers. Furthermore, the current understanding of regulation of obesity by dietary phytochemicals found in fruits, vegetables, and edible plants (e.g., spices) is discussed.

II ROLE OF ADIPOSE TISSUE IN OBESITY

A Adipose Tissue Expansion

1 Hyperplasic Adipocytes

Adipose tissue develops as a result of hypertrophy (i.e., adipocyte size increase), hyperplasia (i.e., adipocyte number increase), or both. Adipocyte hypertrophy plays an important role in regulating the ability of cells' lipid storage and secretion of adipose-specific hormones/factors that contribute to the systemic energy balance, inflammation, and energy homeostasis. On the other hand, adipocyte hyperplasia, termed adipogenesis, is known to contribute to the generation of new adipocytes during childhood and adolescence in humans, as well as adipocyte turnover in adults [2]. This implies that adipogenesis could be as an effective cellular process for prevention of adipose tissue generation and, by extension, the development of

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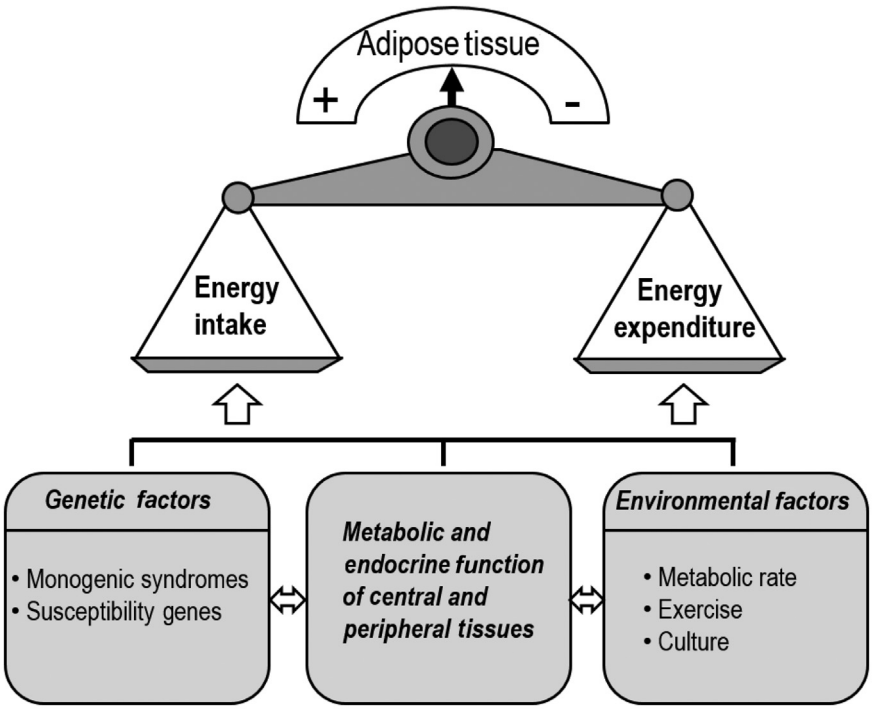


FIGURE 20.1 Factors influencing energy balance.

obesity in young adults. Mesodermal pluripotent stem cell driven preadipocytes undergo adipogenesis when cells are exposed to appropriate hormonal and nutritional signals. Adipogenesis consists of four distinct steps, including (1) early growth arrest of confluent preadipocytes, (2) mitotic clonal expansion (MCE), (3) transcription of adipogenic transcription factors, and (4) terminal cell differentiation [3]. Growth arrest of preadipocytes in the early phase of adipogenesis is coordinately regulated by transcriptional control of genes involved in the cell cycle by adipogenic transcription factors CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ) [3]. Upon exposure to mitogenic and adipogenic signals, the growth-arrested preadipocytes undergo at least one round of cell cycle with DNA replication and cell division. Although MCE appears to be required for promoting adipogenesis of most established preadipocyte cell lines in vitro, it is reported to be not necessary for adipogenesis of other cells such as primary human preadipocytes [3]. Nevertheless, inhibition of MCE by cell cycle inhibitors [4] and modulation of genes encoding cell cycle components [5,6] have been shown to impair adipogenesis. In addition, phosphorylational activation of the adipogenic insulin signaling pathway such as insulin receptor, insulin receptor substrate-1 and -2, phosphoinositide 3-kinase, and protein kinase B/Akt in the early phase of adipogenesis also participates in the promotion of adipogenesis. Some cell cycle regulators such as cyclin D, Rb, and E2Fs [7] appear to link between MCE and the transcriptional events in the early phase of adipogenesis. Concomitant with the MCE process, DNA binding ability of C/EBPβ, an early adipogenic transcription factor, and its transcriptional activity are acquired. This occurs through sequential phosphorylation of C/EBPβ by a cell cycle regulator cdk2/cyclinA, mitogen-activated protein kinase (MAPK), and glycogen synthase kinase-3β during MCE [8]. Phosphorylated C/EBPβ then subsequently induces its target adipogenic transcription factors C/EBPα and PPARγ.

Whereas C/EBPβ-induced expression of C/EBPα is required for promotion of adipogenesis and formation of white adipose tissue, PPARγ is reported to reverse the defective function of C/EBPβ and C/EBPα in adipogenesis [7], suggesting that PPARγ as the central regulator of adipogenesis. PPARγ forms a heterodimer with retinoid X receptor, and this heterodimeric complex binds to the PPAR response element located in the promoter regions of many genes involved in fat storage and adipokine production. Several agonists such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and thiazolidinediones are also known to promote transactivation of PPARγ.

During the terminal phase of adipogenesis, genes encoding enzymes in lipid metabolism such as glycerol-3-phosphate acyltransferase (GPAT), glycerol-3-phosphate dehydrogenase, glyceraldehydes-3-phosphate dehydrogenase, fatty acid synthase (FAS), acetyl coenzyme A (CoA)

carboxylase (ACC), and stearoyl-CoA desaturase (SCD) are dramatically activated. Levels of genes involved in fatty acid update and transport, and lipid droplet formation, including an adipose-specific fatty acid binding protein (aP2), a fatty acid transporter (FAT/CD36), and perilipin, are also increased. In addition, mature adipocytes produce adipocyte-specific hormones such as leptin, adiponectin, resistin, adipsin, and plasminogen activator inhibitor-1, as well as a number of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1α, and monocyte chemotactic protein-1. These adipocyte secreted factors are known to modulate peripheral and systemic energy balance.

Molecular and dietary regulation of adipogenesis is suggested to prevent the body weight gain and the development of obesity. However, it should be noted that inhibition of adipogenesis alone could possibly result in adipocyte hypertrophy and/or redirection of body fat to other nonadipose tissues, which increase the risk of the development of obesity-related diseases. Thus, additional approaches to improve whole-body energy expenditure possibly through activation of thermogenesis and/or fatty acid oxidation should be accompanied by the blockage of adipogenesis.

2 Hypertrophic Adipocytes

Adipose tissue is traditionally viewed as a storage organ of lipids. During approximately the past decade, adipose tissue has been further revealed as an endocrine tissue secreting a number of hormones and factors that modulate systemic glucose and lipid homeostasis. De novo lipogenesis plays a key role in adipocyte hypertrophy, as well as hepatic lipid synthesis. It was reported that the increase in adipose tissue in lean and obese adults is largely due to adipocyte hypertrophy since adipocyte hyperplasia is rather constant in adults [2]. This study indicates that adipocyte hypertrophy could play a key role in the development of adult obesity. The substrates for de novo lipogenesis are fatty acid esters and CoA. Transport of mitochondrial citrate to cytosol by tricarboxylate transporter initiates the generation of acetyl-CoA and malonyl-CoA by ATP-citrate synthase and ACC, respectively, followed by subsequent synthesis of fatty acids, mostly palmitate, by FAS. The most abundant saturated and unsaturated fatty acids found in adipocytes are stearate and oleate, respectively. A single *cis* double bond at the Δ^9 of oleate is introduced by SCD-1. Conversely, mice deficient in ACC [9] or SCD-1 [10] were found to be resistant to adiposity. Similarly, administration of an inhibitor of FAS such as a fungal-derived cerulenin and synthetic C75 promoted long-term weight reduction in obese animals [11,12], suggesting that FAS is an effective target protein for the control of de novo lipogenesis.

Triglycerides are synthesized by esterification of glycerol with three molecules of fatty acids. Thus, glycerol 3-phosphoate, a glycolytic metabolite, and fatty acyl CoAs are the key substrates for triglyceride synthesis. Fatty acids used for triglyceride synthesis are mainly derived from de novo lipogenesis, hydrolysis of triglycerides in the cell, or fatty acids transported from circulation. The first step of an esterification of a fatty acyl CoA to glycerol 3-phosphate is catalyzed by GPAT, and this is followed by subsequent esterification of two fatty acyl CoAs catalyzed by endoplasmic reticulum resident enzymes such as GPAT, *sn*-1-acylglycerol-3-phosphate acyltransferase, phosphatidic acid phosphatase, and diacylglycerol acyltransferase. The newly synthesized triglycerides in the endoplasmic reticulum are then coated by several lipid droplet envelope binding proteins, such as perilipin and adipose differentiation-related protein, which are required for the formation of triglyceride-enriched lipid droplets and an effective mobilization of lipid droplets under energy demanding condition. In supporting of the importance of these enzymes in triglyceride metabolism, genetic deletion of a gene encoding one of these enzymes in mice [13–16] and humans [17–19] with mutation of one of these genes have been shown to lower adiposity and/or energy balance. However, total inhibition of enzyme activities by genetic deletion of their corresponding genes is known to be associated with a number of side effects such as insulin resistance, skin-barrier abnormalities, and/or neonatal lethality [20]. This could be avoided if a selective and moderate inhibition of the function of enzymes involved in triglyceride synthesis could be achieved. In this regard, identification of natural phytochemicals that significantly, but not completely, inhibit activities of enzymes involved in triglyceride synthesis would effectively modulate the development of obesity [21].

Adipokines secreted from differentiated adipocytes regulate energy metabolism in peripheral tissues and brain in a paracrine and endocrine manner. Among the many adipokines, a significant amount of evidence suggests the important functions of leptin and adiponectin in the modulation of lipid metabolism both in adipose tissue and in nonadipose tissues such as liver and muscle. Leptin is a 16-kDa peptide that belongs to the cytokine class 1 superfamily. Leptin is encoded by *Lep* in mice and *LEP* in humans. Leptin exerts its biological functions by binding to its receptor encoded by *Lepr* in mice and *LEPR* in humans. Circulating leptin is correlated with body mass index (BMI), and defects in leptin and leptin receptor genes trigger the development of obesity and diabetes in both humans and animals [22,23]. The activated leptin–leptin receptor signaling pathway in peripheral tissues and in hypothalamus influences lipid metabolism and food intake, respectively. Leptin is known to induce mobilization of triglycerides in lipid droplets (i.e., lipolysis) by

activation of AMP-activated protein kinase (AMPK) and its-dependent fatty acid oxidation in skeletal muscle [24]. On the other hand, leptin is suggested to inhibit triglyceride synthesis and increase lipolysis in adipocytes through activation of leptin signaling pathway and suppression of genes involved in lipogenesis [25]. Although leptin not only appears to directly regulate the function of AMPK and its related triglyceride turnover, but it also indirectly modulates lipid metabolism through interference of insulin-induced lipogenesis and insulin-inhibited fatty acid oxidation, suggesting that leptin functions as a counter-regulatory hormone to insulin.

Adiponectin is a 244-amino-acid hormone mainly secreted from adipose tissue. Recent studies have further identified the expression of adiponectin mRNA in the liver and muscle tissues [26,27]. A number of animal studies indicated that adiponectin promotes fatty acid oxidation and suppresses gluconeogenesis in the liver, thereby promoting energy expenditure and protecting animals from high-diet-induced obesity and insulin resistance [28,29]. Conversely, obesity is associated with a low level of circulating adiponectin. Adiponectin-induced resistance to obesity and improvement of insulin sensitivity occur through interaction between adiponectin and the two receptors—a muscle-specific ADIPOR1 and a liver-specific ADIPOR2 [30]. The adiponectin-ADIPOR axis also appears to have direct and indirect effects on protection from obesity-related diseases such as atherosclerosis, cardiovascular diseases, and nonalcoholic steatohepatitis. These health benefits of adiponectin are exerted largely through allosteric activation of AMPK activity, and transcription of genes involved in fatty acid oxidation such as PPARα and PPARγ. Adiponectin expression and secretion is known to be modulated by various nutritional and inflammatory cues. For instance, fasting-refeeding [31], long-term caloric restriction [32], insulin treatment [33], and PPARγ agonists [34] induce adiponectin production, whereas inflammatory cytokines (e.g., TNF-α and IL-6) [35,36] and glucocorticoid [35] suppress adiponectin production.

During the development of obesity, hypertrophic adipocytes are known to display (i) increased basal lipolysis with elevated level of free fatty acid release, (ii) increased leptin secretion, (iii) deceased adiponectin secretion, (iv) increased expression and secretion of proinflammatory cytokines, and (v) impaired insulin-dependent glucose uptake (Table 20.1). Consequently, adipocyte hypertrophy is associated not only with adipose tissue expansion but also with impaired adipocyte function, thereby aggravating whole-body energy balance. On the other hand, hyperplasic adipocytes with relatively smaller cell size than hypertrophic adipocytes may counteract many detrimental effects of adipocyte hypertrophy in obesity (Table 20.1).

TABLE 20.1 Comparison of Adipocyte Hypertrophy and Hyperplasia in Obesity

Hypertrophy	Hyperplasia
Cell size (increased)	Cell number (increased)
Free fatty acid release (increased)	Free fatty acid release (decreased)
Leptin release (increased)	Leptin release (increased)
Adiponectin release (decreased)	Adiponectin release (increased)
Proinflammatory cytokine release (increased)	Proinflammatory cytokine release (decreased)
Insulin sensitivity (decreased)	Insulin sensitivity (increased)

B Function of Adipose Tissue in Energy Balance

Adipose tissue mass is closely associated with food intake regulation. As an endocrine organ, adipose tissue transmits a signal to the brain for central control of energy balance. Much attention has been given to the effect of an adipose-specific hormone leptin on central food intake. The interaction between leptin and its receptor expressed in hypothalamus is known to coordinately regulate central food intake signaling such as the proopiomelanocortin (POMC)-dependent anorexic pathway and the neuropeptide Y (NPY)/agouti-related protein (AgRP)-dependent hyperphagic pathway. Leptin-induced cleavage of POMC and its subsequent activation of α-melanocyte-stimulating hormone (α-MSH) and melanocortin receptor contribute to a decrease in food intake. On the other hand, reduction of circulating leptin level triggers the release of hyperphagic hormones NPY and AgRP in the hypothalamus. AgRP released under leptin deficiency in turn inhibits the anorexic function of α-MSH, resulting in stimulation of food intake [37,38]. Analogous to leptin, the level of plasma insulin, which is directly correlated with adipocyte hypertrophy, also modulates central food intake through the interaction with insulin receptor expressed in the hypothalamus for controlling anorexic and hyperphagic pathways [38,39]. Collectively, when adipose mass is reduced, low levels of circulating leptin and insulin are known to stimulate food intake and reduce energy expenditure. In contrast, increased levels of these hormones in circulation result in suppression of food intake and increased energy expenditure.

AMPK, a whole-body energy sensor protein, not only controls peripheral energy homeostasis but also integrates nutritional and hormonal signals in the brain to control central food intake and energy balance. Leptin-induced

dephosphorylation and inactivation of the hypothalamic AMPK has been shown to inhibit food intake. AMPK also plays an important role in hypothalamic de novo lipogenesis and its impact on food intake. AMPK activation in the hypothalamus under the insulin deficiency, for example, results in an inhibition of ACC activity with a reduced level of malonyl-CoA, which in turn modulates the function of hypothalamic neurons and increases food intake. On the other hand, inhibition of AMPK activity in the hypothalamus by central administration of glucose [40], metabolic intermediate citrate [41], or α-lipoic acid [42] resulted in the suppression of food intake. Moreover, inhibition of de novo lipogenic enzyme FAS activity by C75 [43] in the hypothalamus resulted in the accumulation of malonyl-CoA with an inhibition of food intake.

Central regulation of energy balance is also attributed by endocrine function of some intestinal hormones. Intestinal hormones such as cholecystokinin [44], peptide YY [45], and glucagon-like peptide-1 [46] are major representatives of endocrine-gut-brain communications that sense the size and frequency of meals in intestine. Thus, elevated levels of the release of these hormones from intestine are known to lower food intake, appetite, and weight gain through interaction with their specific receptors in the brain. Interestingly, some dietary compounds in coffee, such as chlorogenic acid and caffeine [47] and isoflavones [48], are known to modulate the release of intestinal hormones and lower satiety.

III OBESITY-RELATED CANCERS

Obesity is a global health crisis. Nearly 300 million adults are obese worldwide, and approximately two-thirds of U.S. adults are overweight or obese [49]. Obesity has been implicated in a number of chronic diseases, including diabetes and cardiovascular diseases [50]. As one of the life-threatening diseases, cancer has a high mortality rate. Increased cancer incidence has been observed in developing countries, as well as in developed countries, including the United States. Tobacco smoke, including secondhand smoke, exposure to sun, air pollution, exposure to toxic chemicals, infections, alcohol intake, and family history are responsible for cancer development. Obesity is a critical risk factor for developing specific types of cancers, as well. A cohort study estimated that 15−20% of deaths from all cancers are attributable to overweight and obesity in the United States [51]. Epidemiological studies have also suggested that greater BMI is associated with increased incidence and/or death from a few forms of cancer, including colon, breast, esophageal, and endometrial cancers [52]. However, studies of the associations between obesity and the mortality from cancers often yield inconsistent results, and the mechanisms by which obesity mediates tumor formation and progression are not clearly understood.

The World Cancer Research Fund has found that obesity is associated with greater risks from cancers of colorectal, breast (postmenopausal), endometrial, and esophageal, and probably other types of cancers [53]. In obesity, adipose tissue dysfunction is a key contributor to the development of hyperinsulinemia and insulin resistance. Insulin stimulates the production of insulin-like growth factor (IGF)-1 in adipose tissue, where it plays an important role in the cancer development by inducing cell proliferation and inhibiting apoptosis [54]. Binding of IGF-1 to its receptor (IGF-1R) activates multiple signaling cascades, including phosphatidylinositol 3-kinase (PI3K)-Akt and Ras/Raf/MAPK, which in turn lead to cellular growth, proliferation, differentiation, and motility [54].

Another mechanism associated with adiposity and carcinogenesis is the bioavailability of sex steroids [55]. Particularly, estrogen is mainly synthesized from adipose tissue, and its circulating level is directly associated with BMI in postmenopausal women [56]. Increased circulating levels and bioactivity of IGF-1 can decrease hepatic synthesis and circulating levels of sex-hormone-binding globulin (SHBG), which has a high affinity to testosterone and estradiol [57]. Indeed, in both men and women, decreased SHBG levels due to higher adiposity led to increased concentrations of free estradiol and testosterone [57]. After binding to their receptors, estrogen and testosterone can diffuse into the target cells, and subsequently promote cell proliferation and inhibit apoptosis [55].

A Colorectal Cancer

Colorectal cancer is the third most common type of cancer in the world. It is estimated that nearly 9.7% (1.23 million) of all commonly diagnosed cancers worldwide are attributed to colon cancer [58]. An epidemiological study has suggested that obesity is associated with an increased risk of colorectal cancer [59]. A prospective cohort study found direct associations between obesity measures such as waist circumference, waist:hip ratio, body weight, and BMI, and the risk for colorectal cancer in men [60]. Another clinical study performed in Japan found that colorectal cancer is significantly correlated with the accumulation of visceral fat, but not subcutaneous fat [61]. This study suggests that visceral adipose tissue accumulation is likely to be a stronger predictor for colorectal cancer development [61].

Although the mechanisms by which greater adiposity regulates colorectal cancer development are not clearly understood, increased circulating levels of leptin may be associated with obesity and colon cancer. A cohort study indicated that people with high leptin levels had an

approximately 30-fold higher risk of colon cancer [62]. Increased levels of leptin receptors have been found in colon tumors, polyps, and adjacent mucosa, as well as human colon cancer cells [63]. Obese mice also had greater body and epididymal fat mass, and increased levels of serum leptin and leptin receptor. An experimental study showed that obese mice fed a high-fat diet were more susceptible to carcinogen-induced colon tumorigenesis than normal diet fed mice [64]. This study suggests that obesity induced by high-fat diet stimulates colon tumor formation, which is likely mediated by increased levels of circulating leptin and its binding to the leptin receptor. Supporting to this notion, leptin is suggested to stimulate cell proliferation via activation of nuclear factor-κB (NF-κB) and extracellular signal-regulated kinase (ERK)1/2-mediated pathways in colonic epithelial cells [63,65]. Leptin is also known to induce angiogenesis [66], which results in tumor growth, invasion, and metastasis [67]. Moreover, recent study suggested a link between obesity and the silencing of colonic cell surface receptor guanylyl cyclase C (GUCY2C) in colorectal cancer. Their study suggested that caloric suppression of guanylin expression, silencing GUCY2C, disrupts the intestinal epithelial barrier, producing systemic genotoxic stress contributing to extraintestinal tumorigenesis in obesity [68].

B Breast Cancer

Breast cancer is the second most common cancer in the world, and it is the first leading cause of cancer-related mortality in U.S. women [69]. In particular, obesity increases the risk of breast cancer among postmenopausal women partially due to obesity-mediated upregulation of serum-free estradiol [70]. Whereas estrogen is produced mainly in the ovaries in premenopausal women, it is alternatively produced from adipose tissue in postmenopausal women [71]. Hence, the positive association between obesity and the risk of breast cancer among postmenopausal women is likely attributable to greater estrogen production from adipose tissue.

The mechanisms underlying the link between obesity and breast cancer risk are not fully understood. However, the potential mechanisms may involve obesity-mediated alterations in the levels of growth factors (i.e., IGF-1), subsequently modified sex hormones, and elevated levels of inflammatory cytokines and adipokines. Obesity is associated with increased levels of insulin and IGF-1, leading to the downregulation of circulating SHBG, which in turn increases bioavailability of free estradiol [57]. This increased amount of estradiol is considered to be a strong risk factor for the development of postmenopausal breast cancer [71].

Patients with breast cancer exhibited increased concentrations of plasma inflammatory cytokines, such as TNF-α, and IL-1β, -6, and -8 compared to their age-matched controls [72]. Conversely, elevated level of inflammation may also contribute to the increased risk of breast cancer. Indeed, obesity is considered to be a state with chronic low-grade systemic inflammation. Obesity-induced production of inflammatory cytokine TNF-α causes insulin resistance through activation of IκB kinase (IKK) complex, which in turn induces phosphorylation of serine residues of insulin resistance substrate [73]. In addition, other proinflammatory cytokine IL-6 activates Janus kinase (JAK)/the signal transducer and activator of transcription (STAT)-3 signaling pathways which in turn induce cancer cell proliferation [74].

Additionally, the positive relations between obesity and breast cancer development can be explained by obesity-mediated reductions in plasma levels of adipokines [75]. Among breast cancer patients, postmenopausal women exhibited significantly decreased serum adiponectin level compared to premenopausal women [76]. An in vitro study demonstrated that adiponectin suppresses cell proliferation and promotes cell growth arrest and apoptosis in MDA-MB-231 breast cancer cell [77]. The mechanism by which adiponectin prevents cancer progression is through the ADIPOR1 and R2-medicated activation of AMPK. Recent epidemiological studies have found a lower cancer incidence in the patients with type 2 diabetes after treatment with a metformin, an AMPK activator [78], indicating a key role of AMPK in cancer prevention. Interestingly, it was recently reported that obesity promotes cancer progression and metastasis by altering the biomechanical microenvironment of fatty adipose tissue in the breast [79]. Their findings suggest that obesity-induced interstitial fibrosis promotes breast tumorigenesis by altering mammary extracellular matrix mechanics.

C Endometrial Cancer

Endometrial cancer has the highest incidence rate in Western countries [80]. Case-control and cohort studies strongly suggested a positive association between overweight and/or obesity and endometrial cancer risk [51,81]. In particular, childhood BMI and height were shown to be associated with the risk of later development of endometrial carcinogenesis [82].

As with breast cancer, obesity-associated induction of circulating estrogen and reduction of SHBG level is likely to contribute to the development of endometrial cancer [83]. In addition, other studies have also found a close relation between obesity-caused induction of leptin and the risk of endometrial cancer [84]. Leptin has been shown to stimulate proliferation of human endometrial cancer cells through functional activation of cyclooxygenase-2 (COX-2), which is mediated via JAK2/STAT3, MAPK/ERK, and PI3K/AKT signaling pathways [85]. This finding

emphasizes the importance of leptin and leptin-mediated activation of COX-2 in obesity-triggered endometrial cancer development. It was also suggested that p27 is associated with the development of endometrial cancer in obesity. Indeed, loss of p27 was shown to be linked to the risk of endometrial cancer at the early progression stage [86].

D Esophageal Cancer

In addition to alcohol and tobacco uses, obesity is another critical risk factor for esophageal disorders: esophageal cancer, Barrett esophagus, and gastroesophageal reflux disease (GERD) [80], as supported by a recent meta-analysis that included 12 case-control studies and 2 cohort studies [87]. Among these disorders, esophageal cancer and GERD are reported to be positively correlated with obesity indices such as BMI and body fat distribution (i.e., abdominal body fat). Mechanisms underlying the relationship between esophageal cancer risk and obesity are not fully understood, but leptin is also suggested to play an important role in the development of esophageal cancer. Leptin promotes proliferation and mobility of human esophageal cancer cells [54] and this is likely through the activation of ERK and epidermal growth factor receptor (EGFR) [88]. Examination of the relationship between leptin receptor and adiponectin receptor (AdipoR1 and AdipoR2) expression in esophageal cancer and obesity status revealed that AdipoR1 is associated with obesity and is an independent predictor of patient survival [89]. In addition, both esophageal cancer and GERD are associated with systemic inflammation [90,91]. Because obesity is associated with a state of low-grade inflammation, obesity-induced systemic inflammation and elevated levels of secreted proinflammatory cytokines may promote the development of these esophageal disorders.

E Other Obesity-Related Cancers

Several studies have examined the potential relationship between obesity and the risk of other cancers such as bladder, prostate, and pancreatic cancers. However, the findings are not consistent. A case-control study has found a positive association between greater adiposity and the risk of bladder cancer [92]. However, a prospective cohort study of approximately 500,000 men and women has revealed a positive but modest correlation between adiposity and bladder cancer risk [93]. Although more than 40 studies, including various prospective and case-control studies, were conducted to examine the relationship between obesity and prostate cancer risk, the results were conflicting [94]. While several studies have suggested that increased BMI is likely to be associated with about twofold greater risk of pancreatic cancer in both men and women [95], but a meta-analysis using case-control and cohort studies did not find a weak relationship between BMI and pancreatic cancer risk [96]. Thus, whether obesity plays a role in the development and progression of bladder, prostate, and pancreatic cancers still remains inconclusive.

IV PHYTOCHEMICALS IN OBESITY AND ITS RELATED CANCERS

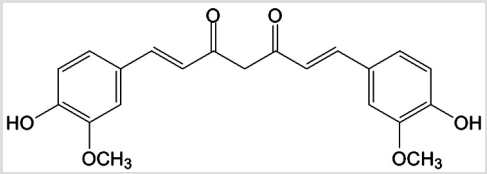
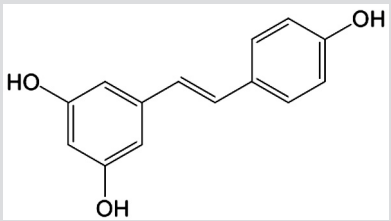
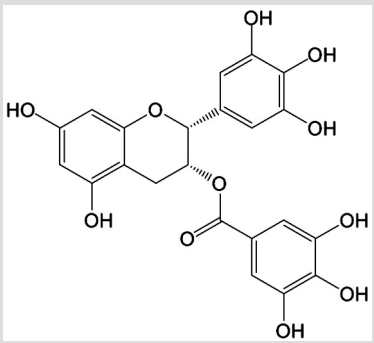
Currently a variety of approaches are used in the treatment of cancer including surgery, chemotherapy, and radiation therapies. While these treatments strive to decrease mortality rates and improve survival rates from cancers, they are associated with several side effects. Furthermore, patients undergoing typical treatment regimens often cannot be completely cured. It is reported that appropriate dietary modification contributes to an approximately one-third decrease in death rates for all cancers in the United States [97,98].

Natural bioactive compounds have been demonstrated to regulate a wide range of cellular and signaling processes in a variety of cancer cell types [99]. Fruits, vegetables, and edible plants (e.g., spices) are good sources of vitamins, minerals, and dietary fiber for basic nutrition. In addition, they are excellent sources of micronutrients, and natural bioactive phytochemicals such as polyphenols, phenolics, and alkaloids, are believed to contribute to many of the health benefits associated with fruits and vegetables. During the past several decades, a great deal of research has focused on characterizing which dietary phytochemicals are most effective in modulation of inflammatory, oxidative, and cell proliferative processes which substantially contribute to the initiation of many human diseases including cancer. Indeed, a number of epidemiological studies support the hypothesis that phytochemicals in fruits, vegetables, and plants reduce the risk of various forms of cancer [100–105]. Whereas many natural phytochemicals have been identified to be chemopreventive, efforts in identification of antiobese phytochemicals and their mode of actions have only more recently begun to emerge. Therefore, natural phytochemicals, which specially have both antiobese and chemopreventive properties, could be promising dietary approaches for the prevention of the development of obesity and its related cancer progression. The dietary phytochemicals reported to exhibit both potential antiobese and chemopreventive properties are summarized in Table 20.2 and discussed later.

A Curcumin

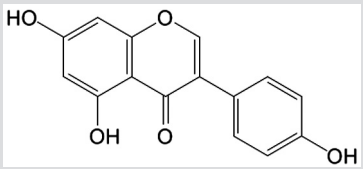
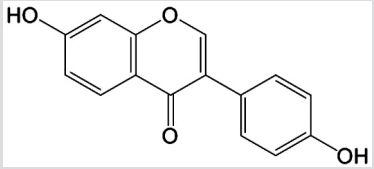
Curcumin (diferuloylmethane) is a natural yellow pigment and derived from turmeric, a powdered rhizome of *Curcuma longa* Linn (Table 20.2). Curcumin's protective

TABLE 20.2 Summary of Molecular Targets and End Point Modulation of Potential Antiobese and Chemopreventive Phytochemicals

Name	Structure	Molecular Targets and End Point Modulation	Reference
Curcumin		Lowered plasma triglycerides and VLDL levels, and liver triglycerides and cholesterol levels	[192]
		Lowered body weight gain, blood glucose and insulin levels	[108]
		Lowered body fat gain	[193]
		Reversed obesity-induced glucose and insulin tolerance	[194]
		Reduced lipid accumulation and inflammation	[114,194]
		Reduced body weight gain, adiposity, angiogenesis	[111–114]
		Activation of AMPK in adipose tissue	[116–118]
		Inhibited adipogenesis	[119]
		Reduced NF-κB activity and cell proliferation	[116,118]
		Induction of apoptosis of cancer cells	
		Inhibition of AP-1 activity	
Resveratrol		Decreased PPARγ expression	[129]
		Induced glucose uptake	
		Reduced de novo lipogenesis	[131,132]
		Induction of apoptosis	[133,134]
		Increased insulin sensitivity, AMPK function, PGC-1α expression	[135]
		Improved mitochondria function	[136,138]
		Reduced adiposity	[138]
		Suppressed leptin	
		Reduced EGFR, IGF1-R	
		Induced apoptosis and p53 signaling pathway	
EGCG		Decreased fat mass	[143,144]
		Reduced levels of serum lipid metabolites and/or body fat	[146–149]
		Increased thermogenesis and energy expenditure	[151–153]
		Inhibited gene expression and/or activity of lipogenic enzymes	[152,195]
		Activated AMPK	[152–157]
		Inhibited adipogenesis and adipocyte function	[161]
		Reduced the levels of IGF-1, IGFBP-3	[163,164]
		Suppressed TNF-α expression and NF-κB activity	[164,165]
		Induced apoptosis	
		Promoted cell cycle arrest	

(*Continued*)

TABLE 20.2 (Continued)

Name	Structure	Molecular Targets and End Point Modulation	Reference
Soy phytoestrogens	Genistein 	Lowered BMI	[174]
	Daidzein 	Improved insulin sensitivity and blood HDL level	[175,176]
		Reduced body and fat weight gain	[177]
		Suppressed de novo lipogenesis	[152]
		Stimulated lipolysis	[177–179]
		Inhibited adipogenesis	[179,180]
		Activated apoptosis and AMPK	[181]
		Inhibited apoptosis	[188]
		Inhibited hepatic triglyceride content and fatty acid synthesis	[189]
		Reduced sex steroid receptor protein expression	[190,191]
		Suppressed NF-κB activity	
		Inhibited ER-α, ER-β, and AR gene expression	
		Suppressed PTK activity	
		Inhibited TGFβ1-mediated cell growth	

activities against various diseases, such as diabetes, obesity, and cancers, have been intensively studied during the past several decades. Accumulating evidence suggested that curcumin exhibits antioxidant, antiinflammatory, anticarcinogenic, and cardioprotective properties [106], which are attributed to modulation of multiple targets in the cells and development of various diseases [99].

Curcumin has been reported to improve the profile of plasma lipid metabolites in obese animals. Curcuminoid-rich extracts [commercial-grade curcumin (~73%) mixed with demethoxycurcumin (~16%) and bisdemethoxycurcumin (~11%)] have been reported to lower liver triglyceride and cholesterol levels, and plasma triglycerides and VLDL fraction in animals fed a high-fat diet [107]. In diabetic mice, curcumin significantly suppressed body weight gain, blood glucose and insulin levels largely through lowering hepatic lipid metabolism [108]. The direct effect of curcumin intake on obesity has recently been addressed by Weisberg et al. who examined the therapeutic effect of curcumin on obese and diabetic animals [109]. Oral administration of 3% dietary curcumin to obese and diabetic mice for 6 weeks exhibited lower body fat gain and reversed obesity-induced glucose and insulin tolerance. Curcumin treatment also reduced lipid accumulation and inflammation in the liver, and macrophage infiltration of adipose tissue.

Curcumin has also been reported to prevent the development of obesity. A long-term feeding study showed that mice with high-fat diet supplemented with a low dose of curcumin (i.e., 500 mg/kg diet) at least for 12 weeks showed a reduction of body weight gain, adiposity, and angiogenesis in adipose tissue without affecting on food intake behavior [110]. This was associated with activation of AMPK, transcriptional suppression of genes involved in lipogenesis and angiogenesis in adipose tissue, and an increase in fatty acid oxidation. In addition, curcumin was demonstrated to have an antiadipogenic property both in vitro [111–114] and in vivo [110] through suppression of C/EBPα and PPARγ gene expression, and MCE, and activation of Wnt/β-catenin signaling, AMPK activity, and apoptosis of preadipocytes in the early phase of adipogenesis. Curcumin was also shown to induce brown fat-like phenotype in 3T3-L1 and primary white adipocytes. In this study, expression of UCP1 and other brown adipocyte-specific genes was found to be increased by

curcumin, which is likely mediated by curcumin-induced activation of AMPK [115].

Although the specific molecular mechanisms underlying curcumin's anticancer activities are not fully understood, several mechanisms underlying the chemopreventive function of curcumin have been proposed. Upon activation of cancer cells by free radicals, inflammatory mediators, carcinogens, or ultraviolet light, a transcription factor NF-κB senses these signals and induces expression of several genes involved in the cell survival, transformation, proliferation, and metastasis [116]. Curcumin is believed to suppress NF-κB activation by inhibiting the DNA binding ability of NF-κB, and reducing the activation of IKK complex, which in turn led to inhibition of inhibitory factor IκB phosphorylation, and subsequent translocation of NF-κB into the nucleus [117]. Curcumin-inhibited NF-κB activation results in the suppression of transcriptions of NF-κB-regulated genes involved in cancer cell proliferation such as COX-2, TNF-α, cyclin D1, adhesion molecules, and metalloproteinases [118]. The resulting impact is induction of apoptosis, programmed cell death. Curcumin has also been reported to promote cell cycle arrest in the G1/S phase in human colon carcinoma cells [119] by downregulating NF-κB-dependent cyclin D1 expression [118]. Curcumin also inhibits activity of a transcription factor activating protein-1 (AP-1). AP-1 regulates expression of several genes involved in apoptosis, cell proliferation, and progression. Importantly, AP-1 promotes tumor metastasis by repressing tumor-suppressor genes, including p53, p21, and p16 [116]. Curcumin suppresses AP-1 activation in the prostate cancer cell lines such as LNCaP, PC-3, and DUI145 [116]. Inhibition of AP-1 transcriptional activity may be attributed to curcumin-mediated inhibition of c-Jun N-terminal kinase phosphorylation [120] that otherwise leads to cell proliferation and tumorigenesis. In lung cancer, curcumin exhibited anticancer effect by suppressing the expression of enhancer of zeste homolog 2 (EZH2) in lung cancer cells which in turn decreased the expression of NOTCH1 [121].

Although curcumin is safe even at higher doses of 2 g/day in humans, the limited oral bioavailability of native curcumin has limited its therapeutic and/or preventive use in human diseases. Poor intestinal absorption of curcumin combined with its rapid hepatic metabolism and physiological clearance are hurdles that have served to limit its effectiveness as a chemopreventative agent in vivo. Developments of liposome, micelle, and phospholipid complex [122–125] with curcumin have long been studied and are promising approaches to improve bioavailability of curcumin. In addition, recently developed nanoparticle-based delivery of curcumin [126] and conjugation of curcumin with polyethylene glycol [111] could improve the bioavailability of curcumin, but the efficacy and safety of these complexes remain to be fully investigated and leveraged in a therapeutic approach.

B Resveratrol

Resveratrol (Table 20.2), a phenolic compound found in a diverse array of plants, has received much scientific interest throughout the years. A naturally occurring stilbenoid found in grapes, berries, peanuts, and sugar cane, resveratrol is best known from its association with the "French Paradox." Resveratrol has been reported to have a therapeutic potential against many chronic diseases. Its reported health benefits include hypolipidemic, chemopreventive, antiinflammatory, and antioxidant activities by largely targeting Sirtuin 1 (Sirt1) [127].

Resveratrol is reported to modulate adipose development and function via regulation of various cellular processes. As a dietary activator of Sirt1, resveratrol decreases lipid accumulation and promotes free fatty acid release from adipocytes through Sirt1-dependent repression of PPARγ function [128]. Resveratrol also inhibits adipogenesis of human adipocytes in a Sirt1-dependent manner through stimulation of basal and insulin-induced glucose uptake with reduced level of de novo lipogenesis in vitro [129]. Recent data showed that resveratrol inhibits lipogenesis of differentiating preadipocytes by inhibiting insulin signaling and mitochondrial biogenesis, and this contributes to adipose tissue weight loss in animals and humans [130]. In addition, Sirt1-independent function of resveratrol in fat cells has been proposed in which resveratrol regulates the number of differentiated human adipocytes through induction of apoptosis [131]. Indeed, resveratrol has been shown to be an effective proapoptotic agent both in preadipocytes [132] and in adipocytes, by which it controls, at least in part, the function and number of adipocytes. Nevertheless, mice administrated with a high-fat diet supplemented with resveratrol at 0.04% (w/w) of diet for 50 weeks [133] or at 0.4% (w/w) for 8 weeks [134] displayed enhanced insulin sensitivity, AMPK function, PPARγ coactivator-1α (PGC-1α) expression, and improvement of mitochondria function as well as reduced adiposity, suggesting a protective function of resveratrol against diet-induced obesity and its related metabolic disorders. However, more studies are needed targeting improved bioavailability of resveratrol to enhance circulating levels and presence in target tissues as a path to better translate these health benefits in humans.

Although the data are still insufficient to reach a conclusion, the protective actions of resveratrol against cancer development are likely attributable to the suppressions of leptin secretion [135]. Leptin acts as an important protein linking between obesity and cancer [62]. Indeed, a high-fat diet increases circulating levels of serum leptin, and leptin treatment has been shown to increase colonic cell proliferation in mice [64] and human colon cancer cells [63]. Given that resveratrol is known to suppress adipogenesis, resveratrol-mediated suppression of leptin secretion in adipose tissue likely contributes to prevent cancer development.

In addition, growth factor receptors are known to play an important role in the development and progression of colon cancer [136] via stimulating cell growth, angiogenesis, and metastasis. Accumulated evidence suggests that resveratrol-suppressed growth factor receptors, including EGFR and insulin-like growth factor-1 receptor (IGF-1R), also appear to mediate its chemopreventive function. In fact, animal experiment with Wistar rats has demonstrated that resveratrol administration for 30 weeks significantly attenuates colonic tumor incidence [137]. Resveratrol has been shown to suppress IGF-1-induced cell proliferation and induce apoptosis in human colon cancer cells by inhibiting IGF-1R/Wnt function and activating p53 signaling pathways [138].

C Tea Catechins

Consumption of teas, including green, black, white, and oolong, is commonly associated with a number of positive impacts on human health. The beneficial effect of consuming tea on body weight management appears to be largely through the modulation of whole-body energy balance. The potential mechanisms by which green tea regulates body weight and adiposity may be attributed to an increase in energy expenditure and fat oxidation, and a decrease in intestinal lipid absorption [139]. A long-term consumption of catechins (also known as flavan-3-ols or flavanols), which are natural phenolic antioxidants found in teas, is associated with reduction in body weight and body fat in humans [140,141]. Dietary catechins include: epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG). In particular, EGCG is the most abundant polyphenols found in green tea (Table 20.2), and it is most effective in preventing the development of various diseases, including obesity and type 2 diabetes [142]. EGCG decreases subcutaneous fat mass by an average of 55% and abdominal fat by an average of 28% in lean rats after 1 week of intraperitoneal injection of a dose of 70−92 mg EGCG/kg body weight [143]. A 4-day EGCG treatment also induced a 20% body fat loss in obese rats [144].

Although the antiobesity function of EGCG in humans is inconclusive yet, a number of human epidemiological studies suggest a possible benefit of EGCG and tea consumption in weight loss [101,103]. Consumption of high levels of green tea (more than 10 cups/day) [145], habitual tea consumption (more than 10 years) [146], and consumption of a bottle of oolong tea containing 690 mg catechins/day for 12 weeks [147] in different human populations resulted in lower levels of serum lipid metabolites and/or body fat, as well as a greater thermogenesis and energy expenditure [148,149]. It is important to note that other components of tea, such as caffeine, are also suggested to contribute, at least in part, to the health benefit of tea consumption [150].

Despite the important health benefit of EGCG, the detailed mechanism of its action in regulating body weight and adipose mass is relatively unclear. In general, EGCG and other catechins are known to inhibit gene expression and/or activity of lipogenic enzymes such as FAS, SCD-1, and glucose-6-phosphate dehydrogenase, thereby reducing fatty acid and triglyceride synthesis in adipose tissue and the liver [151−153] partly through activation of AMPK [152] and/or inhibition of adipogenesis of adipocytes. EGCG also displays a direct inhibitory effect on adipogenesis via regulation of multiple cellular processes and the transcriptional program in adipogenesis. In addition to its antiadipogenic property, EGCG has been reported to exhibit antimitogenic [154] and/or proapoptotic [155] effect, and to act as a transcriptional suppressor [156], and/or AMPK activator [157] during adipogenesis.

Numerous studies have suggested that EGCG may be effective in preventing cancer development [158]. A recent meta-analysis study showed a positive association between increased green tea consumption and a significant reduction of breast cancer risk [159]. An in vivo study using rats fed a high-fat diet supplemented with black tea has also found to have decreased tumor numbers, size, and multiplicity after the administration of a chemical carcinogen [160]. The chemopreventive mechanisms of catechins found in green and/or black tea have been proposed in a number of studies. Catechins appear to inhibit the function of growth factor receptors and their-downstream mediators, and NF-κB activation. Catechins also induce cancer cell apoptosis and cell cycle arrest. Oral administration of green tea polyphenol mixture to the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model has been shown to inhibit the development and progression of prostate tumor by reducing the levels of IGF-1 and IGF-binding protein (IGFBP)-3, and its downstream signaling pathways in dorsolateral prostate [161]. The role of TNF-α is important in carcinogenesis because it acts as a growth factor for most of the tumor cells [162]. However, EGCG has been shown to suppress TNF-α expression [163] through inhibition of NF-κB activity [164]. On the other hand, EGCG induces apoptosis by stabilizing p53 in human prostate carcinoma LNCaP cells [165]. Likewise, promotion of cell cycle arrest through alteration of cell cycle regulatory protein expression by EGCG may be another molecular mechanism by which EGCG blocks carcinogenesis [164].

Antiobesity activities of EGCG have been well-defined. As discussed previously, green tea, and EGCG from tea in particular, has been suggested to decrease body mass and exert hypolipidemic activity. The potential mechanisms by which green tea regulates body weight

and adiposity may be attributed to an increase in energy expenditure and fat oxidation, and a decrease in intestinal lipid absorption [166–169]. Although it is not clear whether EGCG-lowered body weight and adiposity directly contribute to the prevention of the development of obesity-related cancers, based on the aforementioned studies, it is plausible to hypothesize that EGCG-mediated reductions in body and adipose tissue mass could contribute to the decreased risk of the development of various cancers. Hence, more studies are needed to address this hypothesis and to elucidate whether EGCG could be an ideal candidate to protect both obesity and its related cancers.

D Soy Phytoestrogens

Dietary phytoestrogens are reported to benefit human health [170]. The structural similarities between select phytoestrogens and estradiol have driven the notion that phytoestrogens are able to modulate cellular estrogenic processes through binding to estrogen receptors in various cell types. Indeed, phytoestrogens are largely known to have protective function against menopausal disorders, cardiovascular disease, cancer, and osteoporosis [171–173]. Isoflavones are the key phytoestrogens present in soy. Genistein and daidzein are the two most heavily studied forms (Table 20.2). Isoflavones are largely found in soybean and soybean products, and they are tightly associated with soy protein. In the past decade, a number of studies of humans and animals have provided evidence of a potential antiobesity function of soy protein and soy isoflavones including daidzein and genistein.

Consumption of soy isoflavones was associated with lower BMI, and improved insulin sensitivity, and blood high density lipoprotein (HDL) level in postmenopausal women of normal weight [174]. Moreover, soy protein isolate and its hydrolysate effectively reduced weight gain by lowering adipose tissue mass in genetically obese mice [175] and rats fed a high-fat diet [176]. Although the mechanisms underlying the antiobese function of phytoestrogens are largely unclear, the phytoestrogens appear to modulate systemic energy balance by targeting energy metabolism of a variety of cell types, including adipocytes and hepatocytes. Genistein is shown to suppress de novo lipogenesis and stimulate lipolysis in isolated adipocytes [177]. Moreover, genistein is known to inhibit adipogenesis by suppressing C/EBPβ activity [178], activation of AMPK [157], and apoptosis [179] in differentiating preadipocytes. Contrary to genistein's antiadipogenic effect, daidzein, an analogue of genistein, is reported to have proadipogenic property with improved PPARγ-mediated transcriptional activity and insulin-stimulated glucose uptake [180]. Genistein has also been reported to inhibit hepatic triglyceride content and fatty acid synthesis in the liver and adipose tissue [181]. In addition, administration of soy isoflavones to diet-induced obese male rats resulted in a decrease in body weight, and these effects were accompanied with suppressed lipogenesis and adipogenesis, as well as enhanced lipolysis and β-oxidation. This regulatory effect of soy isoflavones was reported to be mediated via inhibition of AKT/mTORC1 pathway [182]. Collectively, these phytoestrogens seem to have favorable actions on energy metabolism that could partly explain their protective and/or therapeutic effect on obesity and its related glucose disorders.

Epidemiological studies have suggested that increased consumption of phytoestrogens may contribute to a decrease in the rates of recurrence and mortality of breast and prostate cancers [183,184]. A cross-sectional study of 100 women, who had been treated for breast cancer and were in remission, reported that two-third of the patients have consumed some types of soy food with average daily intake of 11.6 mg genistein and 7.4 mg daidzein [185]. Among many other soy isoflavones, genistein has been studied extensively due to its abundance in soy foods, and its protective activities against hormone-dependent cancers [186]. Indeed, many animal studies suggest an anticancer property of genistein in prostate cancer. Dietary supplementation with genistein decreased the percentage of TRAMP mice that developed adenocarcinomas of prostate [187]. Consistent with this finding, supplementation with genistein and daidzein resulted in inhibition of chemical-induced prostate carcinomas in F344 rats [188]. Soy isoflavones are known to be converted to equol and 5-hydroxyl-equol by intestinal bacteria, which exhibit biological function that exceeds those of their precursors. Thus, soy isoflavones' antiobesity and anticancer properties are more likely to be attributed to equal-modulated estrogen- and androgen-dependent cellular conditions.

Although the mechanisms by which genistein protects against obesity-related cancer development have not been fully elucidated, downregulation of sex steroid receptor protein expression, suppression of NF-κB activation, and inhibition of tyrosine kinase in cancer cells by genistein and daidzein have been proposed. Because of the structural similarity between isoflavones and endogenous hormone 17β-estradiol, the potential chemopreventive function of genistein and daidzein is likely to be through modulation of estrogen receptor (ER) function and its related signaling pathways, and this could be mediated by the soy isoflavone metabolite, equol. In support, dietary genistein downregulates mRNA expressions of ER-α and -β as well as androgen receptor (AR) in the dorsolateral prostate of Sprague-Dawley rats [189]. This study suggests that dietary genistein may protect against prostate cancer due to its high affinity with sex steroid hormone receptor. These phytoestrogens also play an inhibitory role in the binding between NF-κB and DNA,

and in the protein tyrosine kinase (PTK) activity observed in prostate and breast cells [190]. In addition, genistein is suggested to target various cellular pathways such as transforming growth factor-β1 (TGF-β1) and its downstream pathways [191]. Collectively, the anticarcinogenic actions of genistein and daidzein are likely mediated through modulation of the functions of NF-κB and PTK, and also TGFβ1-dependent signaling cascades mostly in prostate and breast cancer cells, which otherwise are known to stimulate cancer cell proliferation.

V CONCLUSION

During the last several decades there has been a substantial increase in our understanding of the molecular basis of the development of obesity and obesity-related cancers. The identification of specific molecular targets that contribute to the incidence of these diseases provides the ability to screen new antiobesity and/or chemopreventive dietary components. The potential use of specific food components in the dietary prevention of obesity and its related cancers was discussed. Given the poor bioavailability of most of the aforementioned dietary components in physiological condition, further research should focus on developing new methods to enhance the efficacy and stability of these in circulation and/or target tissues. In addition, detailed preclinical studies and human studies should also be performed.

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Chapter 21

Genetics of Nonsyndromic Human Obesity, With Suggestions for New Studies From Work in Mouse Models

Craig H. Warden and Janis S. Fisler
University of California, Davis, CA, United States

I INTRODUCTION

Complex and incompletely defined interactions between environment and genetics determine each individual's height and weight, as well as other human quantitative traits. The result is a population in which individuals vary widely for height and weight, but no one factor can be identified as controlling either trait in most people. In humans, long-term adult weight is relatively stable, as evidenced by the difficulty of sustaining intentional weight loss and the almost automatic return to previous weight following brief periods of overeating. This drive to constancy of body weight is due to both behavioral and physiological alterations that accompany weight change. Convincing evidence of the biological basis of the regulation of body fat stores comes from the identification of dozens of rare single-gene Mendelian mutations and syndromes that result in spontaneous massive obesity or in adipose tissue atrophy.

Most human obesity, however, is not due to mutations in single genes that have overwhelming effects, but is inherited as a complex, multigenic, quantitative trait influenced by many genetic and environmental variables. There are likely to be interactions among genes and between genes and environmental factors such that some alleles of one gene will not cause obesity unless specific alleles of another gene or environmental pressures are also present. Dietary effects on parents and parental genetics, independent of progeny genotype, also exert powerful but indirect effects on obesity. Genetic heterogeneity, where similar phenotypes are caused by more than one gene, and incomplete penetrance of the trait, where not all people with the gene develop the phenotype, also make dissection of complex phenotypes difficult. Expression of an obesity gene may also be age- or gender dependent. Thus, identification of all the genes promoting human obesity has not been, and will never be, a trivial task.

This chapter is not an exhaustive compendium of all things of genetics and obesity. This chapter does not include discussion of congenital lipodystrophies [1] nor rare genetic syndromes that include obesity in the phenotype, such as Bardet–Biedl and Prader–Willi, as these do not seem to contribute significantly to common causes of obesity and are reviewed elsewhere [2,3]. The present chapter is focused on human genetics, thus we do not plan to discuss the many effects of gut microbiota on metabolism and obesity [4]. The present chapter will discuss both monogenic and multigenic obesity, some of the techniques used to discover them, and general principles derived from these studies. We will list and discuss the most important known obesity genes, but we will not attempt to provide an exhaustive catalog of obesity genes (see [5,6]). Genetics is a rapidly progressing field, and knowledge of the genetic basis for obesity is expanding exponentially. Therefore, the reader should use this chapter to understand the most common genes and mechanisms, general ideas for finding more human obesity genes based on what has already been demonstrated in mice, and an appreciation of the wide variety of mechanisms by which genetics influences obesity.

II THE BIG PICTURE—HOW MUCH OBESITY IS DUE TO GENETICS

Genetic epidemiology of human obesity is the study of the relationships of the various factors determining the

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frequency and distribution of obesity in the population. Such studies of obesity are limited in that they do not examine genetic variations and rarely directly measure the amount or location of body fat. However, genetic epidemiology studies do provide information as to whether there is a genetic basis for the trait, whether a major gene is involved in the population, whether inheritance is maternal or paternal, the relative importance of genes and shared or nonshared environment, and whether expression of the trait is gender or age dependent. Genetic epidemiology studies of human obesity employ a variety of designs and statistical methods, each giving somewhat different estimates for heritability of obesity. For a discussion of genetic epidemiology methods employed in the study of obesity, see [7].

The heritability estimates for human obesity are derived from a large number of studies of adoptees, twins, families, or communities. Population or family studies tend to have lower, and twin studies to have higher, heritability for body mass index (BMI). Heritability of BMI has been estimated from adoption studies to be as low as 10% and from twin studies to be as high as 85% [7,8] (Fig. 21.1). In a pediatric twin study, genetic influences contributed 75–80% in the percent of body fat [11]. The heritability for BMI in a study of childhood obesity in a Hispanic population was 40% and, in that study, heritability of diet and physical activity phenotypes ranged from 32% to 69% [12]. By using data from all types of studies, it is estimated that 40–70% of the within population variation in obesity is due to genetic variation [10] (Fig. 21.1). Most studies indicate that familial environment has only a minor impact on obesity.

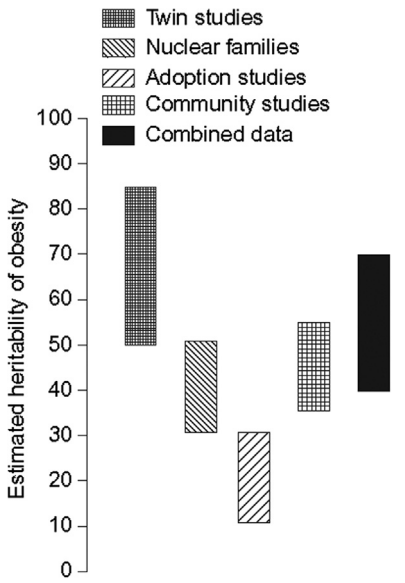

FIGURE 21.1 Heritability of obesity as determined by different study types. *Data for studies of twins, nuclear families, and adoption studies are taken from C. Bouchard, L. Perusse, T. Rice, D.C. Rao, The genetics of human obesity, in: G.A. Bray, C. Bouchard, W.P.T. James (Eds.), Handbook of Obesity, Marcel Dekker, New York, NY, 1998. Data for community-based studies are taken from A. Herbert, N.P. Gerry, M.B. Mcqueen, I.M. Heid, A. Pfeufer, T. Illig, et al., A common genetic variant is associated with adult and childhood obesity. Science 312 (2006) 279–283. Range of heritability estimated from all study types is taken from A.G. Comuzzie, D.B. Allison, The search for human obesity genes. Science 280 (1998) 1374–1377.*

III WHY FINDING OBESITY GENES MATTERS

During 2011–14, in the United States the prevalence of obesity in adults aged 20 years and over was 36% and in youths was 17% [13]. According to the World Health Organization in 2014 of the world population more than 1.9 billion adults were overweight (39%) and of these over 600 million were obese (13%). A total of 42 million children under the age of 5 years were overweight or obese in 2013 (http://www.who.int/mediacentre/factsheets). Obesity rates worldwide are predicted to rise to between 42% and 51% of the adult population by 2030 [14]. If obesity rates were to remain at 2010 levels the savings in medical expenditures over the next two decades could approach $550 billion.

Obesity is not just a financial burden. Sometimes the problems caused by obesity are social, such as discrimination, sometimes obesity influences quality of life by, for example, limiting physical activity, and sometimes obesity is associated with diseases that shorten lifespan, such as heart disease, type 2 diabetes, hypertension, and cancer. Additional obesity comorbidities include arthritis, limitations on mobility, sleep apnea, gallstones, and kidney disease. Until recently there was no way to determine if obesity caused these comorbidities or if they were simply correlated with obesity. Mendelian randomization, a study design that incorporates genetic information into traditional epidemiological methods, now provides a method to determine if genes simultaneously cause both obesity and comorbidity [15]. Causal relations then mean that treatment for obesity becomes even more urgent and that treatment can target specific genetic pathways that cause both obesity and comorbidity.

Studies of people who have lost weight by diet or bariatric surgery prove reduced mortality and improved quality of life. A person's genes influence weight gain, weight loss, and health consequences of obesity. Finding obesity genes may provide tools to improve health of people worldwide.

IV THE SEARCH FOR OBESITY GENES

A Lessons for Human Obesity From Genetic Studies in Mice

Although many decades ago genetic epidemiology studies provided evidence that obesity is highly genetic, there

was no understanding of the molecular basis for obesity until the identification of genes that cause Mendelian forms of obesity in mice. Five genes were known for many decades to cause monogenic obesity syndromes in mice. Positional cloning of the mouse obesity genes, *Lep*^{ob}, *Lepr*^{db}, *Tub*, *Cpe*^{fat}, and *A*^{y}, from naturally occurring mutant models between 1992 and 1996 led to an explosion of knowledge of the genetic causes of obesity [16]. When the third edition of this chapter [17] was published, human orthologs of three mouse obesity genes were known to cause obesity in humans and a fourth mouse obesity gene identified a pathway that caused human obesity. Subsequent studies have now demonstrated that human versions of all five mouse Mendelian obesity genes act in the brain to either directly cause obesity or identify a pathway that causes obesity. These mouse monogenic obesity genes in most instances are recessive and their human orthologs are expected to rarely cause obesity in the human population.

Mouse models of obesity provide information that often replicates causes of human obesity. Several hundred different knockout and transgenic mice have been developed where absence or replacement of a single-gene affects obesity or its phenotypes (for a listing of knockout and transgenic mouse models of obesity and related phenotypes see [6]). These are all possible human obesity genes. These and other genetic studies in mice also show that separate genes control (a) body weight, (b) BMI, (c) sizes of individual fat depots, and (d) responses of individual fat depots to dieting and exercise. Feeding different diets to mice revealed that some mice resist weight gain on diets that make other strains obese, indicating gene−diet interactions. These diet responsive genes are mostly separate from genes for spontaneous obesity on healthy chow diets. Human genetic studies have extensively investigated BMI, have produced smaller studies of overall fatness, but have produced virtually no data on genetics of individual fat depots, and despite many underpowered efforts, almost no significant results on gene−diet interactions.

Mice are valuable in the study of parental effects. Parents may exert indirect effects where female genotype influences progeny phenotype independent of progeny genotype [18,19] possibly by influencing milk composition or quantity, or quality of maternal care. Diets fed to male or female parents may influence weight and health of progeny through epigenetic effects that are heritable changes not due to changes in the underlying DNA sequence. Although similar maternal diet effects are well known in people, the paternal effects in mice have been a surprise as they occur through sperm. Recent evidence from mice suggests that RNA found in sperm can cause obesity and metabolic disorders in progeny of males fed high-fat diets [20]. No comparable studies exist for humans.

Although studies directly in humans have now successfully identified almost 200 obesity genes, taken altogether these account for a limited fraction of heritability and for only a few traits, such as BMI and overall fat distribution. The studies in mice and rats strongly suggest that work to find genes with similar effects on fat depots, diet, and parental effects in humans will identify entire new classes of human obesity genes. Doing so will likely increase the total heritability of obesity that can be explained in humans. Human geneticists have either not explored at all, or only begun to explore, these fundamental aspects of obesity that have been reproducibly demonstrated in mice and rats.

B Identification of Human Obesity Genes by Sequencing

Several approaches are currently used to find new human obesity genes, the most direct of which is to sequence DNA. One can sequence all DNA (whole genome sequencing), or only parts, such as the exome, the protein coding portion of the gene [21]. One recent paper used whole genome sequencing of Sardinians to identify genes influencing height, inflammatory markers, and lipids [22]. Although whole exome sequencing in obesity has been reported [23], no whole genome sequencing studies for obesity have yet been published.

Whether sequencing whole genome or whole exome, most investigators look for genes with mutations that obviously alter function such as stop codons, insertions, or deletions. One of the primary limitations of this approach is that missense mutations that substitute one amino acid for another in genes not previously known to cause obesity tend to be ignored, despite the fact that missense mutations can alter protein function. The practical problem is that each person has many thousands of missense variants and investigators cannot directly test functional effects of all to determine which of these are causal for obesity. Much effort is being devoted to methods to predict which missense mutations in protein coding regions will have functional effects on proteins, but at present there is no substitute for direct studies showing that a missense mutation alters protein function. And since many alleles that cause obesity are not in protein coding regions, ability to predict functional effects of these alleles ranges from nonexistent for alleles far from any gene to sometimes useful predictions for alleles in obvious gene promoter regions. Once again, there remains no convincing substitute for determining direct functional effects.

One exception is that some missense mutations in known obesity genes can be labeled as putative obesity causing. The most common results from sequencing are identification of novel mutations in known obesity genes,

for example, *LEP* [24,25], *LEPR* [24], and *MC4R* [25]. In ideal cases, investigators can show that missense mutations present in obese people will alter the function of a protein. For instance, missense mutations in *MC4R* may alter binding of α-melanocyte-stimulating hormone (α-MSH), localization on the cell surface, or production of cGMP on binding of α-MSH. Currently, mutations have been identified by sequencing in human orthologs of the known obesity genes *LEP* [24,25], *LEPR* [24], *MC4R* [25], *CPE* [26], *TUB* [27], and *PCSK1* [28]. New papers reporting discovery of mutations in these genes occur regularly, so we will not attempt to provide a comprehensive list.

Selected sequencing papers are presented in Table 21.1. Saeed and colleagues found that 30% of severe obesity in children in consanguineous or inbred families was due to variants in *LEP*, *LEPR*, or *MC4R* [24]. Philippe et al. [28] sequenced coding regions of 34 obesity genes in 201 individuals, including 126 who were obese. They report discovery of a nonsense loss of function mutation in *PCSK1* that causes a dominantly inherited familial obesity in a single three-generation pedigree. These investigators [28] also report finding another missense mutation in *PCSK1* and a missense mutation in *POMC* that were previously identified as putative obesity mutations but which are not associated with obesity in their study. This emphasizes the necessity for functional studies of missense mutations for putative obesity genes. Tan et al. [29] provide experimental evidence that exome sequencing did not identify all the obesity-causing mutations in *MC4R*, confirming prior hypotheses that whole genome sequencing will be needed to more completely catalog obesity-causing alleles.

TABLE 21.1 Sequencing Studies of Human Obesity

Gene	Protein Coded	Population	Method (Ref.)	Functional effects of variants confirmed	Comments
Mutations Identified by Sequencing Targeted at Known Obesity Genes					
LEP	Leptin	22 probands from consanguineous families	Targeted sequencing [24]	No	30% of severe obesity in children of consanguineous families due to *LEP*, *LEPR*, or *MC4R*
LEPR	Leptin receptor				
MC4R	Melanocortin receptor 4				
CPE	Carboxypeptidase E	1 individual from consanguineous family	Whole exome sequencing [26]	Yes	Obesity, intellectual disability, abnormal glucose, hypogonadism
TUB	Tubby bipartite transcription factor	1 individual from consanguineous family	Exome sequencing [27]	Yes	Frameshift mutation of *TUB* likely cause of obesity and retinal degeneration in humans Incomplete penetrance
PCSK1	Proprotein convertase subtilisin/kexin type 1	General population 206 of whom 126 obese	Sequenced coding regions [28]	No	One 3-generation family with dominantly inherited obesity
MC4R	Melanocortin receptor 4	267 obese children	Sequenced promoter region of *MC4R* [29]	Yes	Found novel promoter polymorphism that greatly reduced transcriptional activity in 1 child
Novel Obesity Genes Identified by Sequencing					
COA3	Cytochrome C oxidase assembly factor 3	1 obese adult	Whole exome sequencing [30]	Yes	Subject with exercise intolerance, obesity, neuropathy
DYRK1B	Duel specificity tyrosine-phosphorylation-regulated kinase 1B	3 multigenerational families 300 obese	Linkage analysis and whole exome sequencing [31]	Yes	Missense mutation associated with increase of BMI from 23 to 33. Separate variants associated with central obesity and metabolic syndrome

Several recent papers identify novel obesity genes and provide functional data demonstrating that the identified mutations have causal roles. Ostergaard et al. [30] studied a single subject with exercise intolerance, obesity, and neuropathy using whole exome sequencing and found compound heterozygous mutations in cytochrome c oxidase assembly factor 3 (*COA3*). *COA3* is an autosomal gene that is localized to mitochondria with expression highest in metabolically active tissues such as brain, liver, heart, kidney, and small intestine. Thus, effects of this mutation may be tissue specific which may explain the mild phenotype. Keramati et al. [31] used linkage analysis and whole exome sequencing to identify the gene *DYRK1B* as the cause of autosomal dominant coronary heart disease and metabolic syndrome in three multigenerational families. Initial studies identified significant logarithm of the odds (LOD) scores for linkage between markers in *DYRK1B* to BMI, blood pressure, and type 2 diabetes. Whole exome sequencing then identified a missense mutation in *DYRK1B* located in the LOD peak. In the families this mutation is associated with an increase of BMI from 23 to 33. The investigators screened 300 additional obese people and identified a separate variant that was also associated with central obesity. Subsequent letters to the editor by other groups confirmed that yet other missense mutations in *DYRK1B* influence metabolic syndrome.

Sometimes whole exome sequencing does not identify a mutation in a specific gene that causes Mendelian obesity but does identify susceptibility genes that increase risk. Pima Indians have one of the highest incidences of obesity and type 2 diabetes in the United States. They have been participants in, and subjects of, a long running study aimed at discovering if there is a genetic basis for this high incidence of obesity. Whole exome sequencing of 177 Pima Indians identified 31,441 coding variants, none of which had genome-wide significant association with adiposity or measures of type 2 diabetes [32]. A total of 345 of these variants that were predicted to have functional effects were genotyped in additional Pima Indians. *CYB5A* and *RNF10* showed significant association with adiposity and type 2 diabetes but the effects on type 2 diabetes were eliminated when the data were adjusted for BMI. Individuals with the risk allele of *CYB5A* were about 1 BMI unit heavier, while those with the risk allele for *RNF10* were about 3 BMI units heavier. Although these genes are risk factors for obesity, they cannot presently be considered as genes causing Mendelian forms of nonsyndromic obesity.

A small number of papers report the identification of novel genes through whole exome sequencing with possible causal mutations for obesity but do not demonstrate that the mutations identified influence function of the candidate obesity gene. Nevertheless, the utility of the sequence-based approach is likely to grow rapidly because costs are dropping and software for analyzing the results is improving [21]. Several low cost for-profit and nonprofit vendors already offer sequence-based diagnosis to primary care physicians with difficult to diagnose cases. However, most exome sequencing studies do not identify causal genes, and those that do, identify causal genes in only a fraction of the obese people studied. Thus, methods to determine causality of specific mutations remain essential. As discussed in Section IV-A, Lessons for Human Obesity from Genetic Studies in Mice, mouse genetic models often have phenotypes similar to those observed in humans with mutations in orthologous genes. Thus, one method to determine causality is to make the corresponding mutation in mice and then evaluate phenotype. Availability of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 technology means that genetically engineered mice can be made and phenotyped much more quickly than was possible with traditional knockout or transgenic methods [33]. Characterizing the genetic basis of obesity will likely require much more than just exome sequencing.

C Genome-Wide Association Studies—Finding Most of the Common Disease Variants

Starting in 2005, human genetics entered a new era with the introduction of genome-wide association studies (GWAS) that examine many genetic variants in different individuals to see if any variant is associated with a trait. In one of the GWAS several hundred thousand single nucleotide polymorphism (SNP) markers, spread throughout the genome, are used to identify chromosomal regions influencing traits anywhere. GWAS owe their existence to several converging discoveries; sequencing of the human genome, identification of millions of naturally occurring SNPs, and discovery of technologies for determining which allele a person has for hundreds of thousands of SNPs in a single experiment.

One of the key analytical features of GWAS is that investigators do not need to use SNPs that cause disease; they only need to use SNPs that are near the disease-causing allele. More specifically, they need to be in linkage disequilibrium, or close, to the causal alleles. GWAS examine SNPs throughout the genomes of individuals to identify associations between those markers and diseases or specific traits, often comparing genomes of cases (disease) with controls (no disease). GWAS SNP panels are efficient at finding common variants that cause common diseases, but they cannot find rare disease-causing variants. The SNPs used are themselves ones where the minor alleles are relatively common, for instance many

have frequencies of 1−5%, meaning that there cannot be a unique association of any one common SNP with a rare allele. Rare allele discovery requires other methods, such as sequencing. Whole genome association studies have several other disadvantages. In some cases SNPs associated with obesity are located in introns of one gene but appear to act by influencing expression of an adjacent gene. In other cases SNPs associated with obesity are not located within a gene but are between genes.

Many GWAS have been performed for obesity [34,35]. The first obesity locus identified by GWAS was the fat mass and obesity-associated (*FTO*) gene [36], which has significant effects on feeding and on adipose tissue [37]. Although explaining only 1−3% of the variance in BMI, *FTO* polymorphisms have been found in multiple studies of populations worldwide.

The studies with the largest number of patients and providing higher statistical power are meta-analyses from the Genetic Investigation of ANthropomorphic Traits (GIANT) consortium that published several papers in 2015. One paper examined GWAS for BMI in adult men and women [38], while another examined GWAS for waist-to-hip ratio (WHR) after adjustment for BMI [39], and yet another examined the data stratified by gender and age [40].

The BMI study [38] identified 97 genome-wide SNPs, 56 of which were novel and 41 SNPs that had previously been significantly associated with BMI. Table 21.2 lists 13 of the 41 loci with significant association with BMI in multiple GWAS and highlights five of these genes that are components of the leptin-melanocortin pathway. When the data were stratified by gender and age, there was a larger effect in younger rather than older subjects at most of the loci [40]. There were no gender differences.

Estimation of overall heritability explained was conducted in two ways. First, just using genome-wide significant SNPs it was found that about 4% of heritability in BMI was explained. However, using all SNPs in the entire GWAS it was estimated that about 20% of heritability was explained. Of course, this would include some false positives, but may be a better estimate because it includes all the genes with very small effects on BMI.

A total of 35 of the BMI significant SNPs are also identified as associated with other diseases in the National Human Genome Research Institute (NHGRI) GWAS catalog. These include genes that are associated with cardiovascular disease, schizophrenia, smoking, irritable bowel syndrome, and Alzheimer's disease. As we will discuss later, these SNPs and traits are candidates for having causal relationships with BMI in Mendelian randomization studies.

Genes consistently and strongly associated with common obesity (Table 21.2), as measured by BMI in GWAS, include *FTO* (found only in GWAS), *MC4R* (identified as causing single-gene obesity, from sequencing studies, and from GWAS), *TMEM18*, *SEC16B*, and *TFAP2B* (all identified in GWAS), *BDNF* (identified as causing single-gene obesity and from GWAS), *NEGR1* and *FAIM2* (identified from GWAS), *SH2B1* (identified as causing single-gene obesity and from GWAS), and *GIPR* (identified from GWAS) [38,41,43,44].

The *FTO* was first identified through GWAS [45] and consistently shown to be associated with common human obesity across populations and ethnic groups [46]. The function of the FTO protein is unknown although mouse studies suggest it is a 2-oxoglutarate-dependent oxygenase that catalyzes nucleic acid demethylation [47]. *FTO* was recently found to interact with promoters of *IRX3* and *IRX5* that are involved in early neural development and may play a role in adipocyte, especially brown adipocyte, development [48]. *FTO* is highly expressed in the hypothalamus, a primary site for regulation of energy balance and satiety [45]. Risk alleles of *FTO* are associated with increased food intake, increased hunger, and reduced satiety [42], as well as with increased protein intake [49,50]. *FTO* variants interact with fat and carbohydrate intakes to affect BMI [51]. Individuals carrying homozygous *FTO* obesity predisposing alleles may lose more weight through diet or lifestyle intervention than noncarriers [52]. *FTO* variants have also been examined for interaction with physical activity, but the studies to date are not consistent [53].

MC4R, *BDNF*, *SH2B1*, *POMC*, and *TUB*, as components of the melanocortin pathway, contribute to single-gene obesity. However, variants of these genes also contribute to common obesity as measured by BMI in GWAS. Transmembrane protein 18 gene (*TMEM18*) expression levels are related to phenotypes of obesity and glucose metabolism [43,54]. *TMEM18* is widely expressed in the body, both centrally and in adipose tissue. However, its function in energy metabolism is not yet known. *NEGR1* codes for a neuronal growth-promoting factor which may be involved in synaptogenesis, neurite outgrowth, and cell-cell recognition/adhesion [43], and the gene is expressed in the hypothalamus and in peripheral adipose tissue and muscle. The gastric inhibitory polypeptide receptor (*GIPR*) gene codes for a receptor for an appetite-linked hormone, GIP, which is produced in the alimentary tract and mediates enhanced release of insulin from the pancreas. *GIPR* is also expressed in the hypothalamus and adipocytes.

Statistically significant SNPs are rarely causal for common diseases. Locke et al. [38] sought to determine if the significant SNPs were in linkage disequilibrium (close to) coding variants. They found coding variants predicted to have damaging effects on protein function in five genes in linkage disequilibrium with BMI SNPs; *ZNF142*, *STK36*, *TRIM66*, *BDNF*, and *GIPR*. Further study of these variants is needed to determine if they are

TABLE 21.2 Selected BMI Loci Identified in Multiple GWAS (Loci Listed in the Sequence of Strength of Association with BMI) [38]

Notable Gene	Gene Name	Chr	Function	Reference
FTO[a,c]	Fat mass and obesity associated	16	Catalyzes demethylation of RNA. Increased hypothalamic *FTO* expression associated with regulation of energy intake.	[38,41,42]
MC4R[a,b,c]	Melanocortin 4 receptor	18	MC4 protein binds α-MSH and is involved in regulation of feeding behavior and metabolism.	[38,41]
TMEM18[b,c]	Transmembrane protein 18	2	Transcription repressor. Cell migration modulator that enhances the glioma-specific ability of neuronal stem cells.	[38,41,43,44]
SEC16B[b,c]	SEC16 homolog B, endoplasmic reticulum export factor	1	Required for organization of transitional endoplasmic reticulum sites and protein export.	[38,41,44]
TFAP2B[c]	Transcription factor AP-2 beta	6	Transcription factor thought to stimulate cell proliferation and suppress terminal differentiation of specific cell types during embryogenesis.	[38,41]
BDNF[a,c]	Brain-derived neurotrophic factor	11	Helps support growth and differentiation of new neurons and synapses and support the survival of existing neurons.	[38,44]
NEGR1[b,c]	Neuronal growth regulator 1	1	May function as a trans-neural growth-promoting factor.	[38,43,44]
FAIM2[c]	Fas apoptotic inhibitory molecule 2	12	Protects cells from Fas-induced apoptosis.	[38,41]
POMC[a]	Proopiomelanocortin	2	Polypeptide hormone precursor that undergoes extensive, tissue specific, posttranslational processing to produce biologically active peptides, including α-MSH, important in regulation of appetite.	[38]
SH2B1[a]	SH2B adapter protein 1	16	The protein mediates activation of various kinases including *LEP* signaling and other genes of the leptin-melanocortin pathway.	[38,43]
GIPR	Gastric inhibitory polypeptide receptor	19	Stimulates insulin release in the presence of elevated glucose.	[38,43]
POC5	POC5 centriolar protein	5	Essential for the assembly of the distal half of centrioles, required for centriole elongation.	[38,43]
LINGO2	Leucine-rich repeat and Ig domain containing 2	9	unknown	[38,43]
TUB[a]	Tubby bipartite transcription factor	11	Plays a role in obesity and sensorineural degradation	[38]

[a]*Loci associated with genes involved in the leptin-melanocortin pathway. Each of these genes has variants that can cause single-gene obesity in humans.*
[b]*SNPs located near these genes have stronger association with BMI in younger versus older adults.*
[c]*SNPs located near these genes are significantly associated with BMI in children and adolescents.*

causal for the BMI effects. The authors also identified many genes where the BMI SNPs were associated with mRNA levels for adjacent genes, consistent with hypothesis that some of the SNPs influence BMI by altering mRNA levels.

The WHR GWAS, which focused on finding genes for upper versus lower body fat distribution rather than fat mass or mass of individual fat depots, showed several novel features [55]. Shungin et al. [55] found a total of 49 significant SNPs for WHR and another 19 associated with waist or hip circumference measures. Twenty of the SNPs showed strong gender dependence with 19 having stronger effects in women and only a one having stronger effects in men. When stratified for both gender and age, only gender differences were apparent in the data [40]. An additional GWAS paper focusing on adiposity or fat depots is consistent with the GIANT consortium findings. Sung et al. [56] identified multiple SNPs in several genes with gender-specific effects on visceral and subcutaneous adipose tissue.

Alternative measures of obesity may produce different GWAS results. Lu et al. [57] measured percent body fat in 100,716 people by either bioelectric impedance or dual-energy x-ray absorptiometry (DEXA) and found 12 genome-wide statistically significant SNP loci. Seven loci had larger effects on percent body fat than BMI. Five had larger effects on BMI than percent fat. None of the genes was significant for WHR adjusted for BMI. Thus, GWAS contrasting results for BMI and WHR adjusted for BMI, or examining percent body fat or different adipose depots, show different genes for weight and for individual fat depots and substantial genetic differences between males and females. The results are strongly consistent with mouse studies showing that body weight or BMI only partially overlap with percent fat and fat pad genetics.

Other GWAS examined children and various ethnic groups. Studies of children found that most genes for BMI are common with adults and only a few are child specific [41,44]. The results could mean that there are some different genetic controls between adult and childhood obesity, or they could mean that both adult and childhood obesity studies were underpowered and would find the same genes if enough people were studied. Also, most genes in other ethnic groups were the same as those observed in Caucasians; genes that were different may or may not indicate true ethnically different obesity pathways.

GWAS led to the development of genetic risk scores (GRS), multilocus profiles calculated by summing up the number of risk alleles for elevated BMI and obesity. GRS are very useful for studying effects of genetics on response to diet and exercise. They have also been used for the technique of Mendelian randomization, which determines if obesity has a causal effect on correlated comorbidities.

D Mendelian Randomization or Genetic Correlations and Causal Relationships

Obesity is correlated with many diseases, for example, hypertension, type 2 diabetes, cardiovascular disease, serum triglycerides, and more. Until recently there was no method to determine if obesity caused these other diseases, or if one or more other diseases caused obesity. Several methods have just recently become available to identify causal relationships between correlated complex traits. One method looks for genetic correlations. The method does not require individual genotypes, genome-wide significant SNPs, nor even measuring multiple traits for the same individuals. Thus, genetic correlations can be measured for large numbers of traits. Bulik-Sullivan et al. [58] estimated genetic correlations between 24 traits, including BMI. They report positive genetic correlations between BMI and type 2 diabetes, coronary artery disease, and serum triglycerides. They report statistically significant negative correlations between BMI and HDL cholesterol, age at menarche, height, and years of education. These results, and limitations, are quite similar to those observed using the technique of Mendelian randomization. Note that they did not have data on fat mass or fat distribution so comparisons of genetic correlations of BMI with fat mass or fat distribution were not possible.

Mendelian randomization combines genetics with traditional epidemiologic methods to provide causal information about correlated phenotypes, without conducting a randomized controlled trial. The Mendelian randomization approach limits both confounding and reverse causality errors, but assumes there is no linkage disequilibrium or pleiotropy where one gene has a primary effect on more than one phenotype.

A number of recent studies used Mendelian randomization to determine if obesity, as measured by BMI, is causal for disease. Using GRS generated from BMI risk alleles, Todd et al. [59] found evidence that obesity is causal for diabetic kidney disease in type 1 diabetes and Cole et al. [60] found evidence that obesity is a causal risk factor for coronary artery disease. On the other hand, Davies et al. [61] found little evidence that genetically determined BMI and height had influence on prostate cancer risk, but were associated with increased mortality in low-grade disease. Furthermore, Nordestgaard et al. [62] concluded that, although high coffee intake was correlated observationally with low risk of obesity, metabolic syndrome, and type 2 diabetes, there was no evidence to support a causal relationship.

Observational studies suggest that a leptin surge in the perinatal period may program the long-term risk of obesity. A study by Allard et al. [63] using DNA methylation levels near the *LEP* locus in a Mendelian randomization study supports causality between maternal hyperglycemia and epigenetic regulation of leptin in the newborn.

We expect to see more studies using Mendelian randomization to determine causal relations between obesity and disease and between environmental factors and obesity.

E The Problem of Missing Heritability

As much as 70% of any one person's risk for being obese may be heritable, that is, genetic. Yet, to date, less than 10% of that heritability has been identified. The gene most strongly associated with common human obesity, the fat mass and obesity-associated gene (*FTO*), only

contributes about 1% of the variance in BMI [36]. The problem of missing heritability is true not only for obesity but also for most common genetically complex traits and diseases. For example, height is estimated to be 80–90% heritable, yet large-scale population studies identify less than 10% of height's heritability [64].

This missing heritability may be due to the presence of rare variants, variants with low penetrance, copy number variants, epigenetic tags, numerous variants with small effects, or may be due to overestimation of heritability due to epistasis (gene–gene interaction), none of which are readily identified by GWAS.

Rare variants are likely to be missed by the GWAS approach despite the fact that they may have larger effects than common variants [65–67]. Rare variants with large effects seem to be more common in people with early onset or more severe obesity [68,69]. Indeed, sequence-based studies have already found that cohorts with extreme phenotypes of obesity are enriched with highly penetrant but rare alleles. It is likely that sequencing will continue to discover rare variants.

Copy number variation (CNV) is when the number of copies of a particular gene varies among individuals. CNVs comprise more total nucleotide content than SNPs and may encompass one or more partial or entire genes. Yet, in current SNP analytic methods homozygous (A/A), hemizygous (A/O), and duplicative (A/A/A) tend to be lumped. One study of CNVs showed that deletion at the 16p11.2 locus resulted in altered satiety response and subsequent obesity in children whereas duplication at that locus was associated with leanness [70]. Taking account of the structural dimension of the genome might recover some of the missing heritability for many traits [71,72].

Epigenetics refers to changes in gene expression that are not the result of DNA sequence, but can be the result of chemical modifications of DNA or of proteins that bind DNA. Common epigenetic tags are those resulting from DNA methylation or deacetylation of DNA-binding histones. These often occur as the result of early life environment. Evidence now suggests that epigenetic changes are important contributors to inheritance of obesity phenotypes. Since standard sequencing or GWAS techniques do not measure epigenetic tags, they may be a major component of missing heritability. Epigenetics will be discussed in more detail in Section V-A, Epigenetics.

Estimates of heritability assume that there are no gene–gene interactions (epistasis), a phenomenon found in all animal models of obesity. Epistasis is where a gene (or genes) masks or amplifies the effects of another gene (or genes). A classic example of the effect of gene–gene interactions on a complex trait comes from mouse studies. The severity of diabetes in both *Lep*^{ob} (leptin) and *Lepr*^{db} (leptin receptor) mutant mice is determined by the genetic background upon which the mutation is expressed. There are many different inbred mouse strains. These strains differ from each other at millions of places through their genomes. Thus, if the same mutation is moved from one strain to another by breeding, then one can determine if all these other variants influence the phenotypes produced by the mutation. Both *Lep*^{ob} and *Lepr*^{db} mutations in C57BL/6 strain mice result in hyperinsulinemia and obesity, whereas these mutations in C57BLKs strain mice result in severe diabetes and early death [73]. This means that genes other than *Lep*^{ob} and *Lepr*^{db} have dramatic effects on the phenotype that is observed. Characterization of epistasis influencing polygenic obesity in the BSB mouse model, produced by breeding C57BL/6J with *Mus spretus* (a different mouse species) F1 × C57BL/6J, found interaction between genes on different chromosomes that contributed significant variation to obesity and related phenotypes [74]. Each BSB mouse is genetically unique and weights, fat distribution, and fat mass vary widely from mouse to mouse. Quantitative trait locus mapping demonstrated both direct genetic effects and epistatic effects [74].

Gene–gene interactions are likely a universal phenomenon in common human diseases and may be more important in determining the phenotype than the independent main effects of any one susceptibility gene [75,76]. Zuk et al. [77] argue that a high proportion of heritability for certain traits could be due to genetic interactions. Gene–gene interactions are difficult to identify using traditional genetic studies in humans and studies searching for interactions of any gene with all genes are lacking for human obesity. Instead, investigators have performed more limited studies searching for epistasis of pairs of specific genes chosen by investigators. Gene–gene interaction effects have been shown on BMI and waist circumference [78], extreme obesity [79], abdominal fat [80], and on immune dysfunction in obesity [81]. Gene–gene interactions among variants of the β-adrenergic receptor genes (*ADRB1*, *ADRB2*, and *ADRB3*) contribute to longitudinal weight changes in African and Caucasian American subjects [82]. Epistasis affecting obesity was also found in African derived populations in Brazil where interactions between *LEPR* and *ADRB2* polymorphisms as well as a third-order effect between *LEPR*, *ADRB2*, and *INSIG2* were found [83]. In the study of Feitosoa et al., blood lipid profile and dietary habits were found to have confounding effects in the analysis [78]. It is possible to have both gene–gene and gene–environment interactions affecting the same pathway.

Zuk et al. [77] describe a method for estimating heritability not inflated by genetic interactions, but the method requires isolated populations.

V GENE–ENVIRONMENT INTERACTIONS

A Epigenetics

Epigenetics is the study of mitotic and/or meiotic changes due to environmental factors that switch genes on and off without changes in the DNA sequence [84]. Common epigenetic changes result from DNA methylation or histone deacetylation and often occur as the result of environmental exposure in utero, in the early neonatal period or early in life [85]. Epigenetic processes include genome imprinting, gene silencing, and noncoding microRNA, among other effects. Technically, the term epigenetics applies to only those changes that are stably inherited. However, the term epigenetics is also commonly used to describe processes that have not been shown to be heritable but that effect the development of the organism. There is increasing evidence that epigenetics is a mediator of gene–environment interactions underlying the development of obesity and comorbidities [86–90].

Nutrition and activity levels can both affect metabolism through epigenetic gene regulation. Studies that show epigenetic changes associated with weight regulation include studies of the Dutch winter famine that occurred in 1944 showing that children conceived during famine were small and underweight with increased risk for obesity and type 2 diabetes as adults [91,92]. In the Chinese famine of 1958–61, only females developed obesity in later life [93]. DNA isolated from individuals decades after the famine showed abnormal DNA methylation [94]. Studies of identical twins with discordant BMIs identified DNA methylation and expression differences in subcutaneous adipose tissue that distinguish one twin from the other, differences that tended to increase with diverging life experience suggesting that the difference in obesity is epigenetically regulated [95].

Epigenetic studies of human obesity are just beginning. A large-scale epigenome-wide study found significant associations between DNA methylation and BMI and waist circumference in European Americans, which was then replicated in two independent populations including both European and African Americans [96].

Studies showing inheritance of epigenetic changes have been done in rodents. Increased maternal energy intakes affect the epigenetic changes of rats [97]. Feeding a high-fat diet to female mice resulted in increased growth and insulin insensitivity in the progeny [98]. Wei et al. [99] found that prediabetes in male mice increased susceptibility to diabetes in progeny through gamete methylation changes. Mice with the Agouti viable yellow obesity mutation given dietary methyl group supplements have epigenetic changes that prevent passage of obesity to subsequent generations through the agouti viable yellow allele [100]. Integrating mouse to human approaches will be essential to the understanding of the epigenetic contribution to the current obesity epidemic.

B Genetic Effects on Weight Gain or Loss Due to Diet or Exercise

Why some people in modern societies become obese, despite considerable effort and expense to avoid this condition, whereas others stay lean without such effort, appears to have a genetic basis [101,102]. Chronic overfeeding studies by Sims and colleagues beginning in the 1960s showed interindividual differences in weight gain [103,104]. More recently, Bouchard and colleagues determined the response to changes in energy balance by submitting pairs of monozygotic twins either to positive energy balance induced by overeating [105] or to negative energy balance induced by exercise training [106]. Significant intrapair resemblance was observed for changes in body composition and was particularly striking for changes in regional fat distribution and amount of visceral fat. One explanation for these differences is that some twin pairs were better oxidizers of lipid, as evidenced by reduced respiratory quotient, during the submaximal work than were the other twin pairs [106].

Recent epigenome-wide association studies may help explain some of these interindividual differences in body weight response to diet or exercise [107,108]. Young men with low birth weight, suggesting in utero undernutrition, were compared to men of normal birth weight both on a control diet and after 5 days of a high-fat diet. There was no difference in skeletal muscle DNA methylation between low and normal birth weight cohorts on the control diet. However, after the high-fat diet the normal weight group had widespread skeletal muscle DNA methylation, whereas the low birth weight group had few methylation changes [108]. In obese adolescents DNA regions were differentially methylated consistent with weight loss response to diet [109].

Several papers now report DNA methylation changes as a result of acute [110] or chronic [107] exercise training including altered DNA methylation patterns in adipose tissue of healthy young men in candidate genes for obesity including *FTO*, *GRB14*, and *TUB* [107]. A meta-analysis of 10 studies found that individuals carrying homozygous *FTO* obesity predisposing allele lose more weight through diet or lifestyle intervention than noncarriers [52]. These studies are consistent with the hypothesis that exercise may modify DNA methylation, and thus gene expression, for many genes that influence BMI, fat mass, or fat distribution.

Other studies examined genes in the lipolysis pathway for influence on weight loss success but results are not consistent (see [111] for review).

C Genetic Effects on Weight Loss Due to Bariatric Surgery

Several studies with small numbers of subjects report on weight loss in individuals with *MC4R* mutations following bariatric surgery. Patients with *MC4R* mutations are able to lose as much weight as those without such mutations with bariatric surgery in children [112], adolescents [113], and adults [114]. GWAS-type studies with small numbers of subjects are just beginning to look at SNPs associated with weight loss success following bariatric surgery and need confirmation. At the present time, obesity GRS does not predict weight loss results following bariatric surgery [115].

VI GENETIC PATHWAYS OF OBESITY

The hypothalamus is of great importance in obesity as it integrates peripheral hormonal and neuronal signals of satiety and nutritional status, senses nutrients, controls glucose homeostasis and peripheral lipid metabolism, and functions to control whole body energy balance. Much of this control is through the leptin-melanocortin pathway in the hypothalamus. Recent studies also implicate adipose tissue as important in obesity, both as an active endocrine organ [116,117] and in regard to body fat distribution [55].

A The Leptin-Melanocortin Pathway in the Hypothalamus

The first five causal human obesity genes were identified using the mouse models Lep^{ob}, $Lepr^{db}$, *Tub*, Cpe^{fat}, and A^{y}. Once these genes were identified in mice, geneticists began searching for obese humans with mutations in these same genes. Study of the yellow obese A^{y} mouse led to the discovery of the leptin-melanocortin pathway, the primary pathway in the brain which functions in the regulation of body weight (Table 21.3). Searching in highly

TABLE 21.3 Single Gene Mutations Causing Uncomplicated Obesity in Humans and Confirmed in Mouse Models

Gene Name	Chromosomal Transmission	Function of Gene Product Relative to Obesity	Mouse Ortholog
Components of the Leptin-Melanocortin Pathway of the Hypothalamus			
Leptin (*LEP*) [118]	7q31.3 Recessive	Hormone secreted from adipocytes that plays a critical role in regulation of body weight by inhibiting food intake and stimulating energy expenditure. Deficiency causes hyperphagia, early onset obesity, hypogonadotropic hypogonadism, and altered carbohydrate metabolism.	Lep^{ob} cloned from the ob/ob mouse [154]
Leptin receptor (*LEPR*) [119]	1p31 Recessive	Receptor for the hormone leptin. LEPR deficiency causes same phenotype as LEP deficiency.	$Lepr^{db}$ cloned from the db/db mouse [120]
SH2B adaptor protein 1 (*SH2B1*) (155,156)	16p11.2 Recessive	Adaptor protein enhances intracellular leptin signaling in the brain. Loss of function mutation results in hyperphagia, childhood onset obesity, disproportionate leptin resistance, and reduced height as adult.	*Sh2b1* No obesity identified in homozygous null mice.
Proopiomelanocortin (*POMC*) [121]	2p23.3 Recessive	Located in centrally projecting neurons that contain peptide products of proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART). POMC is a precursor protein that is ultimately cleaved into ACTH, α-MSH, β-MSH, γ-MSH, and β-endorphin. Mutation causes hyperphagia, early onset obesity, hypocortisolism, and skin and hair hypopigmentation.	*Pomc* [157]
Tubby bipartite transcription factor (*TUB*) [27]	11p15.5 Recessive	Functions as a membrane-bound transcription regulator in the hypothalamus that translocates to the nucleus in response to phosphoinositide hydrolysis. Deficiency results in hyperphagia, obesity, altered glucose metabolism, and sensorineural degradation.	*Tub* cloned from the tubby mouse [158]

(*Continued*)

TABLE 21.3 (Continued)

Gene Name	Chromosomal Transmission	Function of Gene Product Relative to Obesity	Mouse Ortholog
Carboxypeptidase E (*CPE*) [26]	4q32.3 Recessive	Involved in the synthesis of most neuropeptides and peptide hormones. Deficiency results in severe obesity, type 2 diabetes, intellectual disability, and hypogonadotrophic hypogonadism.	Cpe^{fat}, cloned from the fat mouse [159]
Melanocortin 4 receptor (*MC4R*) [122,123]	18q22 Dominant	The encoded protein is a membrane-bound receptor and member of the melanocortin receptor family, interacts with adrenocorticotropic and MSH hormones, and is mediated by G proteins. Defects in this gene are a cause of hyperphagia, early onset obesity, increased height, and fasting hyperinsulinemia. MC4R mutations cause the most common form of human monogenic obesity ~ 5%.	*Mc4r* [160]
Proprotein convertase subtilisin/kexin type 1 (*PCSK1*) [161]	5q15-q21 Recessive	PC1/3 is a neuroendocrine convertase encoded by *PCSK1* that cleaves POMC and also proinsulin to insulin. Mutation causes hyperphagia, early onset obesity, hypogonadism, and altered carbohydrate metabolism.	*Pcsk1* mutation in the HRS/J inbred mouse results in late onset obesity
Melanocortin 2 receptor assembly protein 2 (*MRAP2*) [162]	6q14.2 Recessive	Regulates energy homeostasis through signaling of MC4R and PKR1.	*Mrap2* knockout produces severe early obesity [163]
Components of the Paraventricular Pathway of the Hypothalamus			
Brain-derived neurotrophic factor (*BDNF*) [124]	11p13 Recessive	Plays a role in the growth, maturation, and maintenance of cells in the brain and is active where cell-to-cell communication occurs. BDNF protein is found in regions of the brain that control eating, drinking, and body weight. Mutation causes hyperphagia, early onset obesity and cognitive impairment.	*Bdnf* [164]
Neurotrophic tyrosine kinase, receptor, type 2 (*NTRK2*) ([165,125])	9q22.1 Recessive	Receptor for BDNF. Mutation results in early onset obesity, hyperphagia, and developmental delay.	*Ntrk2* knock-in results in adiposity [166]
Single-minded homolog 1 (*SIM1*) ([167,126])	6q16.3 Recessive	Transcription factor that is essential for the development of the PVN of the hypothalamus. Haploinsufficiency causes hyperphagia, early onset obesity, altered carbohydrate metabolism, dysmorphic features, and mental retardation.	*Sim1* heterozygous mutants exhibit hyperphagic obesity [168]

consanguineous families including obese individuals, a mutation in the leptin (*LEP*) gene was discovered [118] and confirmed in additional homozygous *LEP*-deficient patients [25]. As with the *LEP* gene, homozygous leptin receptor (*LEPR*) deficiencies were found in severely obese siblings [119] and in families where severe obesity segregated with mutations in *LEPR* [127,128]. Individuals homozygous for mutations in either *LEP* or *LEPR* are hyperphagic and gain weight rapidly in the first year of life [118,129] and have delayed puberty due to hypogonadotropic hypogonadism [128]. Heterozygotes for *LEP* and *LEPR* mutations have increased fat mass but are not morbidly obese.

Borman et al. [130] identified a homozygous mutation in *TUB* in a child of consanguineous marriage. The 11-year old was identified with mild obesity and a 2-year history of deteriorating vision. Functional studies demonstrated that the mutated protein is expressed at low levels in the retina. Alsters et al. [131] identified a homozygous mutation in carboxypeptidase E (*CPE*) in a severely obese woman from a consanguineous marriage. The proband had severe obesity, intellectual disability, abnormal

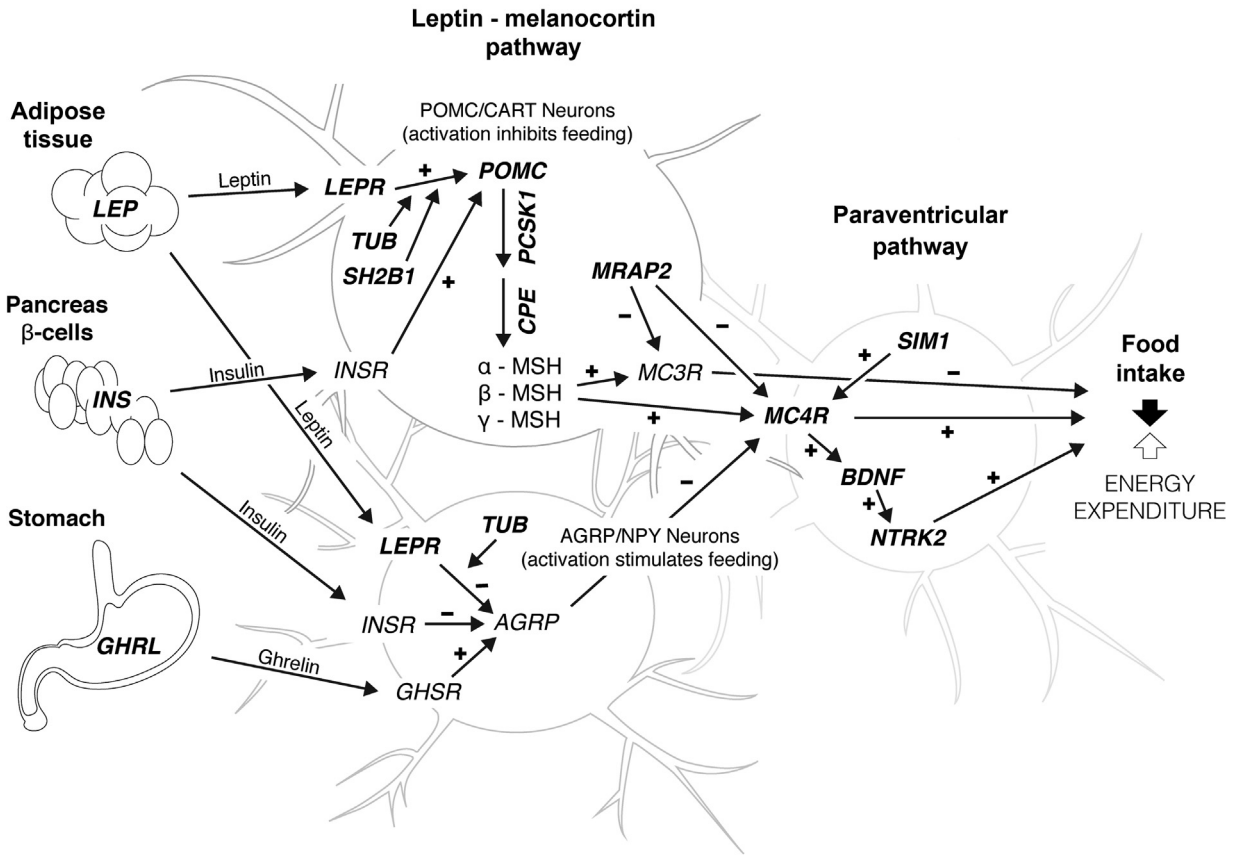

FIGURE 21.2 Simplified schematic of the genes involved in the leptin-melanocortin and paraventricular pathways in the hypothalamus. Mutations of genes in bold are known to cause monogenic obesity in humans. These pathways are essential components of the central control of energy homeostasis, propagating the signals that result in satiety and increased energy intake. Leptin is secreted from adipocytes, crosses the blood–brain barrier and activates leptin receptors. This activation, mediated by tubby bipartite transcription factor, stimulates POMC/CART neurons producing melanocortins that activate melanocortin 4 receptors in the paraventricular and ventromedial nuclei, resulting in satiety. *SIM1* is essential for development of the paraventricular nucleus. Brain-derived neurotrophic factor and its receptor, neurotrophic tyrosine kinase receptor, type 2, are part of the MC4R cascade leading to satiety. When stimulated by ghrelin receptors in the arcuate nucleus, agouti-related protein inhibits MC4R activity. AgRP, agouti-related protein; BDNF, brain-derived neurotrophic factor; CART, cocaine- and amphetamine-related transcript; CPE, carboxypeptidase E; GHSR, ghrelin receptor; INSR, insulin receptor; LEP, leptin; LEPR, leptin receptor; MC3R, melanocortin 3 receptor; MC4R, melanocortin 4 receptor; MRAP2, melanocortin 2 receptor assembly protein 2; α-MSH, α-melanocyte-stimulating hormone; β-MSH, β-melanocyte stimulating hormone; NPY, neuropeptide Y; NTRK2, neurotrophic tyrosine kinase receptor, type 2; PCSK1, proprotein convertase subtilisin/kexin-type 1; POMC, proopiomelanocortin; SIM1, single-minded homolog 1 of *Drosophila*. *Figure illustration by Venus Nguyen.*

glucose, and hypogonadotropic hypogonadism. The proband's symptoms closely match those of Cpe^{fat} mice.

To date, mutations in 12 genes, all components of the leptin-melanocortin or paraventricular pathways, have been reliably shown to cause spontaneous Mendelian increased BMI in humans; leptin (*LEP*), leptin receptor (*LEPR*), tubby bipartite transcription factor (*TUB*), SH2B adapter protein 1 (*SHR2B1*), proopiomelanocortin (*POMC*), proprotein convertase subtilisin/kexin type 1 (*PCSK1*), carboxypeptidase E (*CPE*), melanocortin 2 receptor assembly protein 2 (*MRAP2*), melanocortin 4 receptor (*MC4R*), brain-derived neurotrophic factor (*BDNF*), neurotrophic tyrosine kinase receptor type 2 (*NTRK2*), and single-minded homolog 1 (*SIM1*) (Table 21.3; Fig. 21.2). Mutations in these genes in humans are recessive, with the exception of *MC4R*, and therefore are rare, are associated with hyperphagia and severe obesity beginning in childhood, and may include developmental, endocrine, and behavioral disorders. (For a description of the most common single-gene obesity disorders and syndromes, see [132].)

Leptin (product of *LEP*) is secreted by adipocytes and its concentration in blood is proportional to fat mass. Leptin crosses the blood–brain barrier and activates leptin receptors (product of *LEPR*) on the surface of neurons in the arcuate nucleus of the hypothalamus. This activation, with the assist of the tubby bipartite transcription

factor (product of *TUB*) and SH2B adapter protein 1 (product of *SH3B1*), stimulates proprotein convertase (product of *PCSK1*) and carboxypeptidase E (product of *CPE*) to cleave proopiomelanocortin (product of *POMC*) into the melanocortins including α-MSH, the primary ligand for melanocortin receptors and activation of downstream signaling to regulate energy balance. (For reviews of the leptin-melanocortin pathway and downstream signaling in obesity see [6,133,134].) Activation of the agouti-related protein (AgRP)/neuropeptide Y (NPY) neurons in the hypothalamus stimulates feeding. Leptin binding inhibits the AgRP protein, thereby inhibiting feeding. Thus, leptin functions as an afferent signal in a negative feedback loop to maintain constancy of body fat stores.

Leptin acts through the leptin receptor, a single-transmembrane-domain receptor of the cytokine-receptor family [120]. The leptin receptor is found in many tissues in several alternatively spliced forms, raising the possibility that leptin affects many tissues in addition to the hypothalamus.

Leptin clearly has a broader physiological role than just the regulation of body fat stores. Leptin deficiency results in many of the abnormalities seen in starvation, including reduced body temperature, reduced activity, decreased immune function, and infertility. (For reviews of the physiological role of leptin see [135−137].) Leptin deficiency results in severe hyperphagia and early onset obesity. Replacement with human recombinant leptin in children with severe leptin deficiency normalizes food intake and body composition [136]. Studies of long-term replacement therapy in patients with congenital leptin deficiency show that leptin regulates many body functions including the endocrine system, energy balance, the adipoinsular axis, inflammation, and immunity [138].

Sequential cleavage of the precursor protein proopiomelanocortin (product of *POMC*) generates the melanocortin peptides adrenocorticotrophin (ACTH), the MSHs (α-, β- and δ-MSH), and the opioid-receptor ligand β-endorphin (for review see [139]). α-MSH plays a central role in the regulation of food intake by the activation of the brain melanocortin 4 receptor (product of *MC4R*). The dual role of α-MSH in regulating food intake and influencing hair pigmentation predicts that the phenotype associated with a defect in *POMC* function would include obesity, alteration in pigmentation (e.g., red hair and pale skin in Caucasians), and ACTH deficiency. The observations of these symptoms in two probands led to the identification of three separate mutations within their *POMC* genes [121]. Another *POMC* variant in a region encoding β-MSH results in severe early onset obesity, hyperphagia, and increased linear growth, a phenotype much like that seen with mutations in *MC4R* [140]. Heterozygosity for a *POMC* mutation having subtle effects on proopiomelanocortin expression and function was shown to influence susceptibility to obesity in a large family of Turkish origin [141].

A wide variety of hormones, enzymes, and receptors are initially synthesized as large inactive precursors. To release the active hormone, enzyme, or receptor, these precursors must undergo limited proteolysis by specific convertases. An example is the clipping of proopiomelanocortin by proprotein convertase subtilisin/kexin type 1, also known as prohormone convertase-1 (product of *PCSK1*). Mutations in *PCSK1* were found in individuals with extreme childhood obesity and elevated proinsulin and proopiomelanocortin concentrations but very low insulin levels (for review see [28]). Carboxypeptidase E (CPE) then removes a single basic amino acid from the C-terminus of many different hormones. For example, ACTH is produced from POMC by action of proteases, including PCSK1, then an intermediate product is produced by another protease, and finally α-MSH is produced when CPE removes a final C-terminal amino acid from this last intermediate. A recessive mutation of the gene producing carboxypeptidase E causes obesity in the Cpe^{fat} mouse. Since the human cases and the Cpe^{fat} mouse share similar phenotypes, it can be inferred that molecular defects in prohormone conversion represent a generic mechanism for obesity.

Several melanocortin receptors are highly expressed in the hypothalamus. Mutations in *MC4R* are found in various ethnic groups and cause the most common form of monogenic obesity in humans. The global presence of obesity-specific *MC4R* mutations is estimated to vary from 2% to 7% among population groups [139,142]. *MC4R*-linked obesity in humans is dominantly inherited with incomplete penetrance. Homozygotes have been observed in consanguineous families and have more severe phenotypes than heterozygotes. Subjects with *MC4R* deficiency are obese from an early age. Adrenal function is not impaired but severe hyperinsulinemia is present in the *MC4R*-deficient subjects. Sexual development and fertility are normal. Affected subjects are hyperphagic and have increased linear growth, similar to what occurs in heterozygous *Mc4r*-deficient mice. *MC4R*-deficient humans also have increased lean mass and bone mineral density and mild central hypothyroidism. Female haploinsufficiency carriers who have only a single functioning copy of *MC4R* are heavier then male carriers in their families, a pattern also seen in *Mc4r*-deficient mice. These data are strong evidence for dominantly inherited obesity, not associated with infertility, due to haploinsufficiency mutations in *MC4R*.

MC3R, while not known to cause single-gene obesity, acts as an autoreceptor indicating the tight regulation of the melanocortin system in energy balance. MC3R modifies energy balance by decreasing feed efficiency. Mutations in *MC3R* are not as common as in *MC4R* and

do not result in an autosomal dominant form of obesity, but may be important contributors to susceptibility to obesity. Two variants of the *MC3R* gene interacted with diet to affect weight loss success in an Italian clinic treating severe childhood obesity [143].

B The Paraventricular Pathway

Three genes important in downstream signaling of the melanocortin system have also been shown to cause single-gene obesities. Single-minded homolog 1, product of *SIM1*, is a regulator of neurogenesis and is essential to the development of the paraventricular nucleus of the hypothalamus [126]. Brain-derived neurotrophic factor, product of *BDNF*, and its receptor, neurotrophic tyrosine kinase receptor type 2, product of *NTRK2*, are involved in signaling in the ventromedial nucleus of the hypothalamus and contribute to memory and learning [125]. *BDNF*-deficient rodents are hyperphagic and obese. Case reports associate mutation in *BDNF* or *NTRK2* with massive obesity and impaired cognitive function [124].

C Genetic Pathways Involved in Common Obesity

Locke et al. [38], using data from all loci significantly associated with BMI in the GIANT Consortium GWAS meta-analysis, examined the data using pathway analysis. Biochemical analysis identified several gene sets with significant enrichment; neurotrophin signaling, general growth and patterning, basal cell carcinoma, acute myeloid leukemia, and hedgehog signaling. Pathway analysis showed that genes expressed in the nervous system were particularly enriched in the BMI GWAS, with genes expressed in the immune and hemic systems second most abundant. Genes for monogenic obesity, hypothalamic function, and energy homeostasis were frequently observed. Pathway analysis provided "strong support for a role of the central nervous system in obesity susceptibility and implicated new genes and pathways, including those related to synaptic function, glutamine signaling, insulin secretion/action, energy metabolism, lipid biology and adipogenesis" [38].

D Genetic Pathways Involved in Body Fat Distribution

Fewer studies have looked at body fat distribution by GWAS and, except for the GIANT study, sample sizes have been limiting. However, the GIANT consortium data showed that there was little or no overlap between genes associated with BMI and genes associated with WHR.

Pathway analysis also demonstrated that most WHR genes are expressed primarily in adipocytes and adipose tissue. Lack of evidence for association with brown adipose tissue and other adipose depots is likely due to absence of data for these traits. Using predefined gene sets Shungin et al. [55] observed enrichment for vascular endothelial growth factor (*VEGF*), phosphatase and tensin homolog (*PTEN*), insulin receptor (*INSR*), and peroxisome proliferator activated receptors (*PPAR*s). PPARs regulate expression of genes involved in, among other things, adipocyte differentiation, lipid metabolism, and energy balance. Pathway analysis implicated adipogenesis, angiogenesis, transcriptional regulation, and insulin resistance as processes affecting fat distribution. Of note, there was no overlap of these pathways with those identified for BMI.

VII CLINICAL IMPLICATIONS OF THE DISCOVERY OF OBESITY GENES

A Identification of Monogenic Causes of Obesity

Until recently, only the rare Mendelian syndromes, such as Prader–Willi and Bardet–Biedl, were known to cause heritable obesity. These disorders are easily recognized, both by a wide spectrum of phenotypes [132,144] and by the use of cytogenetics assays that are widely available. However, the Mendelian, nonsyndromic obesity disorders are not so easily diagnosed, because obesity is often the only apparent phenotype and clinical assays for known obesity gene mutations are rarely practical. It is estimated that 2–7% of morbidly obese patients have mutations in *MC4R* [122,123,145,146] and an unknown, but smaller, percent have mutations in other obesity genes, including *POMC* [147] and *NTRK2* [125]. Thus, only about 1 in 10 morbidly obese patient has a known mutation that explains the obesity, and molecular assays for the currently known Mendelian obesities would be negative in the majority of morbidly obese patients. Also, there are many known distinct mutations in each of these genes. Thus, no clinical laboratories yet provide diagnosis of these mutations, rather they have only been diagnosed by research laboratories. However, inability to make specific molecular diagnosis does not mean that one cannot identify people with increased risk for genetic obesity, and this may influence choices or approaches to treatment.

Several criteria can be used to estimate the probability that an individual's obesity has a genetic cause (Table 21.4). At the present time, due to the lack of data, these estimates do not produce any quantitative values revealing individual risk that obesity is monogenic, but rather just generic classification, such as likely genetic, uncertain, and likely not genetic.

TABLE 21.4 Suggestions to Evaluate Suspected Monogenic Etiology of Severe Obesity[a,b]

Phenotype	Phenotype Indicative of Genetic Etiology
Characteristic of All Genetic Obesities	
Family history	Having first-degree relatives with severe obesity
Age of onset	Normal birth weight but age of onset of obesity before age 10
Hyperphagia	Hyperphagia developing within first year of life
	Aggressive food-seeking behavior
Phenotype Associated with Specific Gene Mutation	
Very low leptin levels	Mutation in *LEP*
Hypogonadism, delayed puberty, lack of growth spurt	Mutation in *LEP* or *LEPR* or *PCSK1*
Disproportionate insulin resistance	Mutation in *SH2B1*
Developmental delay	Mutation in *SH2B1* or *SIM1*
Low ACTH or high proinsulin levels	Mutation in *POMC* or *PCSK1*
Frequent infections	Mutation in *POMC* (ACTH), *LEP* or *LEPR*
Defective prohormone processing	Mutation in *CPE* or *PCSK1*
Red hair segregating with obesity	Mutation in *POMC*
Severe hyperinsulinemia, acanthosis nigricans	Mutation in *MC4R*
Accelerated linear growth, increased bone mass	Mutation in *MC4R*
Delayed language skills, impaired short-term memory	Mutation in *NTRK2* or *BDNF*

[a]*Data adapted from ([169,170,155,161], [172], [171,128,132,148]).*
[b]*For a complete algorithm for the assessment of a severely obese individual, see [132].*
BDNF, brain-derived neurotrophic factor; *LEP*, leptin; *LEPR*, leptin receptor; *SH2B1*, SH2B adapter protein 1; *SIM1*, single-minded homolog 1; *MC4R*, melanocortin 4 receptor; *NTRK2*, neurotrophic tyrosine kinase receptor, type 2; *PCSK1*, proprotein convertase subtilisin/kexin-type 1; *CPE*, carboxypeptidase E; *POMC*, proopiomelanocortin.

Factors indicating a genetic basis for obesity are: (1) a family history of obesity is consistent with the presence of an obesity gene shared among family members; (2) early age of onset and extreme obesity indicate a genetic basis for obesity; and (3) children with single-gene obesity are normal weight at birth but severe early hyperphagia, often associated with aggressive food-seeking behavior, results in rapid weight gain, usually beginning in the first year of life. Severe obesity in children has been variously defined as a standard deviation score for BMI of more than 2.5 [148] or 3 [128] relative to the appropriate reference population. Extreme trait values are more likely to be genetic for many complex diseases, simply because extremes tend to result from the actions of severe mutations or from mutations in genes that have larger effects [149].

At present, a few diagnostic tools are available for the medical evaluation of patients suspected of having monogenic obesity. The only screening tests available are for those mutations that cause endocrine abnormalities. Serum leptin should be measured. Very low or very high serum leptin levels will indicate mutation in *LEP* or *LEPR*, respectively. However, lack of very high leptin levels cannot rule out homozygous mutations in *LEPR* [128]. A subset of obese individuals has inappropriately low leptin levels for their fat mass, suggesting a less severe defect in leptin regulation [150]. ACTH and proinsulin should be measured to indicate defects in *POMC* or in prohormone processing. Insulin should be measured to evaluate the appropriateness of the degree of hyperinsulinemia as this may indicate an *MC4R* mutation.

Physical appearance provides evidence of *POMC* mutations or the syndromic obesities. *POMC* defects can cause red hair and obesity [121], although most red hair results from mutations in melanocortin 1 receptor (*MC1R*) [151], which does not influence obesity. Thus, red hair is only informative when red hair, ACTH deficiency, and obesity cosegregate within a family.

Prader–Willi, Bardet–Biedl, and other syndromic obesities can be diagnosed by a variety of characteristic

phenotypes, such as small hands and feet, polydactyly, and mental retardation as well as by cytogenetic assays. Thus, one should rule out these diagnoses by phenotype determination and by absence of characteristic chromosomal abnormalities.

B Personalized Treatment Based on Genotype

At present the impact of genetics on diet effectiveness has been the subject of many papers, but all current studies have severe limitations. First, there are some large longitudinal or cohort studies that have reported statistically significant diet−genotype interactions. However, diet-based correlations have yet to provide evidence that stands the test of time. For example, correlations formed the basis for advice to avoid cholesterol and saturated fat, which have rarely been supported by randomized controlled trials. Second, all current randomized controlled trials are underpowered for genetics and thus find no or few significant results. Third, the underlying diet studies test too few diets for too short a time. Not even one large, well-powered study has examined diet−genotype interactions for diets that range from ketogenic to low carbohydrate or the typical U.S. diet to extreme low fat and vegan. Many basic questions are thus unanswered. For instance, does each person have one ideal diet for weight management or many possible equally healthy diets?

Matching diets to genotype is a goal for personalized medicine. Goals of personalized medicine are sometimes called P4; predictive, preventative, personalized, and participatory. The ability to calculate GRS is now well established but surprisingly, GRS may not predict weight gain or loss. Other components of P4 are not as advanced. Diet predictions based on questionnaire are flawed because diet questionnaires are unreliable. If people are resistant to trying new diets on their own, will they also resist when some professional or expert says "your obesity GRS means that you should be eating…?" One recent study reported that subjects told that they have higher genetic risk alleles of *FTO* had enhanced readiness to control weight but the knowledge of *FTO* status had no impact on behavior [152].

A 2016 NIH Working Group Report [153] on using genomic information to guide weight management pointed out that technologies are available for the fast characterization of the transcriptome, proteome, epigenome, and metabolome of an individual. But effective algorithms are yet to be developed to combine these data with classical medical and behavioral measures of the individual to personalize weight loss recommendations in the clinical setting.

Despite the ability to generate overwhelming amounts of genetic and other data for people, P4 recommendations for diet cannot be implemented. It is not known which of the many natural variants detected matter, nor do the diet studies needed to evaluate variants for causal effects on diet−genotype interactions exist. Thus, for a long foreseeable future, individuals will need to determine optimal diets by personally testing several different diets. The first step toward generalized discovery of personalized diets will require large highly powered randomized diet studies testing a full range of diets.

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Chapter 22

Obesity: Overview of Medical Treatments and Interventions

Kenya D. Palmer and Caroline M. Apovian
Boston University School of Medicine, Boston, MA, United States

I INTRODUCTION

Obesity has reached pandemic proportion, despite efforts to reduce obesity since 2000 [1]. In the United States, almost 70% of the adult population is either overweight or obese, 35% of which are obese [2]. As of 2014, 1.9 billion adults are overweight, including more than 600 million adults who are obese; and 42 million children (<5 years old) [3]. Adult women are more effected by obesity than men; and minorities (black and Hispanic) more than whites [1]. Hispanic men are more likely to be overweight (80%) and obese (38%) than any other racial demographic, and non-Hispanic black women are more likely to be overweight (82%) and obese (58%) [4]. It is believed that with the current rate of growth, obesity will continue to increase to greater than 50% of the adult population [5].

Obesity is associated with several comorbid conditions, including type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension (HTN), cardiovascular disease (CVD), stroke, gallbladder disease, obstructive sleep apnea (OSA), cancer, and osteoarthritis; as well as increased risk for all-cause and CVD mortality [6]. Considering the comorbid conditions associated with obesity it is not unimaginable that obesity would also be a costly disease. Although the economic burden of obesity is difficult to assess, given the comorbid conditions such as T2DM, it is possible that it costs the United States over $200 billion annually [7]. Given the health and financial consequences, obesity should be and is now considered a disease by the American Medical Association (AMA) [8].

Obesity is a chronic disease affecting adults and children of every race, socioeconomic and educational category. Every day those with obesity struggle to lose and maintain weight loss [9]. Given the comorbid diseases and potential complications, it is important for the health care provider (HCP) to understand how to approach and treat these patients successfully. Thus, the purpose of this chapter is to provide an overview of the clinical interventions available for the treatment of obesity. We will begin by taking the reader through the clinical assessment of the patients who are overweight and obese and evaluation of health risk.

II ASSESSMENT OF OVERWEIGHT AND OBESITY

The U.S. Preventative Services Task Force recommends screening all adults over the age of 18 years old for obesity [10]. The first guidelines for the management of obesity were developed in 1998 by the National Heart, Lung, and Blood Institute (NHLBI). Since 2010, several organizations have reevaluated how the adult with obesity is assessed clinically. In 2013 the American Heart Association (AHA), American College of Cardiologists (ACC) and The Obesity Society (TOS) gathered to update the NHLBI guidelines creating the "Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report" for the management of adults with overweight and obesity [6]. Additionally, the American Association of Clinical Endocrinologist and the American College of Endocrinologists collaborated, developing the "Complications Centric Model for Care of the Obese Patient" [11]. Finally, in 2015, the Endocrine Society released clinical practice guidelines

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TABLE 22.1 BMI, WC, and Cardiometabolic Disease Risk

BMI Classification			Cardiometabolic Disease Risk[a] (Relative to Normal Weight and WC[b])	
	BMI (kg/m^2)	Obesity class	Men (<102 cm)	Men (>102 cm)
			Women (<88 cm)	Women (>88 cm)
Underweight	<18	–	–	–
Normal weight	18–24.9	–	–	–
Overweight	25–29.9	–	–	High
Obesity	30–34.9	Obesity, Class I	High	Very high
Obesity	35–39.9	Obesity, Class II	Very high	Very high
Extreme obesity	>40	Obesity, Class III	Extremely high	Extremely high

[a]*Disease risk for developing T2DM, HTN, and CVD.*
[b]*Elevated WC also denotes increased risk, even in normal weight individuals.*
Source: M.D. Jensen, et al., 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society, Circulation 129 (2014) S102–S138.

outlining the clinical approach to the assessment of patients with obesity [12].

The guidelines, regardless of the organization, call for the HCP to begin assessing patients based on their body mass index (BMI) and waist circumference (WC). Further assessment involves determining patients' actual, or risk for comorbid, condition. Treatment recommendations are based on the compilation of the aforementioned characteristics of the patient.

A Clinical Assessment of Body Fat

Obesity is not just excess weight, but an excess of body fat. Historically, obesity was defined according to the Metropolitan Life Insurance Company "ideal body weight" tables based on mortality data [13]. Given the controversy surrounding the utilization of obesity measures based on nongeneralizable data, new assessment tools were determined [14].

1 BMI and WC

Prior to 1985, the Metropolitan Life Insurance Company height–weight tables were used to identify health hazard based on ideal weight [15]. BMI, a closer estimation of body fat, has since replaced these ideal height–weight tables. Overweight is defined as a BMI 25–29.9 kg/m^2; and obesity as a BMI greater than 30 kg/m^2, which is further stratified into classes (Class I BMI 30–34.9 kg/m^2; Class II BMI 35–39.9 kg/m^2; Class III or extreme obesity: BMI greater than 40 kg/m^2) [6].

This measure and WC are recognized as the most convenient and expedient ways to identify body fat, morbidity, and mortality both in population-based research and the clinical setting. We know there is increased health risk associated with increased BMI (Table 22.1). Berrington de Gonzalez et al. [16] reported that in nonsmoking men and women, there is an increase in all-cause mortality with each 5 kg/m^2 increase in BMI; specifically, 30% in women (overweight, hazard ratio (HR) 1.13; obesity Class I, HR 1.44, CI 95%; obesity Class II, HR 1.88, CI 95%; obesity, Class III, HR 2.51, CI 95%). Similar risk increases were seen in men, however, the increase was greater than 30% for obesity classes II and III [16]. WC assesses risk for cardiometabolic disease in individuals with BMI 25–34.9 kg/m^2 [6]. It is assumed that in those with BMI greater than 35 kg/m^2 WC will likely be greater than 88 cm in women or 102 cm in men; and thus adds very little to the assessment of cardiometabolic risk.

Unfortunately, there are some variations in BMI and WC that make utilizing these measurements alone, inadequate [15]. When defining obesity according to percentage of body fat, %bf (25% in men, 30% in women), BMI often underestimates %bf in those with a BMI under 30 kg/m^2 [17] and may overemphasize body fat in women and minorities, making BMI most appropriate when gender and race of the individual is not considered [18]. Because of the greater prevalence of obesity among minorities, it is important that these discrepancies are not ignored.

Sharma and Kushner [15] suggest taking into consideration other individual attributes in addition to BMI and WC to guide clinical judgment for the treatment of obesity. The Edmonton Obesity Staging System (EOSS) (Table 22.2), an ordinal classification system, which

TABLE 22.2 Edmonton Obesity Staging System

Edmonton Obesity Staging System			
Stage	**Medical**	**Mental**	**Functional**
0	Absent	Absent	Absent
1	Preclinical/risk factors	Mild	Mild
2	Comorbidity	Moderate	Moderate
3	End organ damage	Severe	Severe
4	End stage	End stage	End stage

Source: Adapted from J.L. Kuk, C.I. Ardern, T.S. Church, A.M. Shamra, R. Padwal, X. Sui, S.N. Blair, Edmonton Obesity Staging System: association with weight history and mortality risk, Appl. Physiol. Nutr. Metab. 36 (2011) 570–576.

assesses comorbidity, functionality, and quality of life [15] in an effort to predetermine mortality [19], is a valuable adjunct to BMI and WC [19,20]. As the prevalence of obesity continues to grow, it is important that the HCP has a reliable way of assessing risk in this population. Although BMI and WC are widely accepted in the clinical setting, it is important to consider such tools as the EOSS when determining overall morbidity and mortality in the obese patient given the comorbidity.

B Assessment of Comorbid Conditions and Risk Factors

Obesity is a disease of excess fat. The fat cell, adipocyte, is a dysfunctional proinflammatory endocrine cell which leads to the cardiometabolic diseases associated with obesity. Even before obesity was recognized as a disease by the AMA, there was a known link between obesity and other diseases leading to a weight loss goal which is now increasingly relevant to the long-term survival of our population. The following is not a comprehensive review of all known comorbid conditions and risk factors associated with obesity, but some of the more common.

1 Cardiometabolic Disease Risk

a Type 2 Diabetes Mellitus

T2DM, characterized by hyperglycemia, insulin resistance, and/or impaired insulin sensitivity, affects over 300 million people worldwide and possibly 12–14% of adults in the United States [21]. T2DM may have the greatest association to obesity when compared to any other cardiometabolic disease [22]. The Nurses' Health Study and the National Health and Nutrition Examination Survey III (NHANES III) have provided a great deal of information regarding the relationship between T2DM and obesity. As BMI increases $>35\ kg/m^2$, the relative risk for T2DM increases significantly. This increased risk is closely related to the increase in visceral fat, as measured by WC [23].

Like obesity, T2DM is more prevalent among minorities (15.9% American Indian/Alaskan; 13.2% non-Hispanic blacks; 12.8% Hispanics) than non-Hispanic whites (7.6%) [24,25]. Over the past 20 years, both have increased in prevalence. Data collected from the Behavioral Risk Factor Surveillance System shows the rise in obesity and T2DM paralleled each other from 1990 to 2000, both increasing by almost 50% [22]. As HCP we know that diabetes, especially when uncontrolled, leads to poor long-term health outcomes. Given the relationship between the two, it is important that the HCPs (1) recognize this relationship and (2) provide the appropriate counseling to help patients lose weight and decrease their risk for developing diabetes.

b Hypertension

HTN, defined as a systolic blood pressure (SBP) greater than 140 and/or a diastolic blood pressure (DBP) greater than 90 is related to increased and elevated BMI. HTN is also most prevalent among minorities, specifically non-Hispanic blacks (42.4% men, 44% women) [26]. It is well-known that an increase in body weight is associated with increased HTN and other factors associated with heart disease [27]. Specifically, HTN is associated with weight at adulthood, weight gain throughout adulthood and obesity [28]. The rise in HTN risk as it is associated to weight gain is dose-dependent. This means the greater the weight gain, the greater risk (hazard ratio, 2.52; CI 95%), specifically among men [29]. Just as weight gain negatively affects BP, weight loss positively affects BP. We also know that weight loss is associated with decreased risk for HTN [28]. Weight loss can improve BP, which has been supported by countless studies on weight loss including pharmacotherapeutic randomized control trials. Losing as little as 5 kg can reduce both SBP and DBP, in a dose-dependent fashion [30].

c Cardiovascular Disease

BMI is strongly associated with risk factors such as T2DM, HTN, and dyslipidemia thereby increasing the risk of CVD [4,31]. CVD, including coronary heart disease (CHD) and stroke, poses great health risks for the patient with obesity, and is often complicated by T2DM, HTN, and dyslipidemia. The obese population will likely experience more T2DM (18.5%), HTN (35.7%), and dyslipidemia (49.7%) than their overweight or normal weight cohorts, according to NHANES data [4]. Obesity is a significant independent long-term predictor of CVD and weight gain after adulthood is associated with increased risk regardless of comorbid risk factors such as smoking

[32]. Long-term (26 years) data from the Framingham Heart Study revealed that a 10% increase in weight could increase the risk of developing CVD by 80% in men and 90% in women [32].

Obesity and CVD go hand in hand and there is increased mortality in those who live with both diseases. In discussing the relationship between these two diseases, it is important to mention the "obesity paradox" discussed by [33], as this may create some confusion in how to treat obesity in the presence of CVD. There is international research (Israeli) which has shown those with normal BMI and CHD/CVD are at greater risk for mortality than the mildly obese (BMI 30−35 kg/m^2) whereas those with moderate obesity (BMI >35 kg/m^2) have the expected increased mortality rate. Despite these unexpected findings, one should not forget that both T2DM and HTN risk increase with increased BMI thereby increasing risk of CVD; thus the HCP should not undervalue the benefits of weight loss [33]. As HCP we must appreciate the compounding effects of obesity when CHD, HTN, and T2DM are present. Data from the Framingham Heart Study revealing the relationship between BMI, HTN, and development of T2DM over 30 years leads us to understand the importance of early recognition of obesity and these comorbid conditions [34] and thus early treatment.

2 *Noncardiometabolic Disease Risk*

a Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is hepatic steatosis without cause of secondary hepatic accumulation of fat, further defined based on presence of hepatocellular injury with or without fibrosis or inflammation, which may ultimately lead to cirrhosis [35,36]. Just as population increases in obesity were noted, so have there been increases in the prevalence of NAFLD. From 1998 to 2008, the prevalence of NAFLD doubled (5.5−11%, respectively) [37]. Similar to obesity, NAFLD is most common among minorities, specifically Hispanics (45%) [38].

This hepatic metabolic disease, associated with insulin resistance, is most commonly seen in patients with diabetes and obesity; and may have a 100% prevalence in patients with Class III obesity [36]. Because of its relationship with increased insulin resistance, potentially atherosclerosis, and obesity, NAFLD is yet another weight-related comorbidity the HCP should be aware of when caring for the patient with obesity [22]. Weight loss is recommended as first line treatment for NAFLD by The American Association for the Study of Liver Diseases, the American College of Gastroenterology, and the American Gastroenterology Association [39]. In addition to improvements in liver enzymes alanine aminotransferase and aminotransferase with 4% weight loss [40], evidence suggests 75% remission of NAFLD with 5% weight loss [41].

b Obstructive Sleep Apnea

OSA is a syndrome characterized by repetitive episodes of upper airway obstruction occurring during sleeping hours. Obesity is the primary risk factor for OSA and more than 30% of the obese population has OSA, the majority of which have Class III obesity [42−44]. Although the relationship between OSA and obesity is recognized, causation is unknown. It is possible that obesity causes OSA, but it may be that OSA leads to obesity in some, as we know sleep deprivation is associated with weight gain [45,46]. An increase in weight of >10 kg also increases the risk of increasing severity of OSA, whereas weight loss reduces OSA severity [47].

c Polycystic Ovarian Syndrome

Polycystic ovarian syndrome (PCOS) is an endocrine disorder of reproductive health common among women of childbearing age, associated with chronic anovulation, infertility, and hyperandrogenism. Not only are these women suffering from reproductive abnormality, but they also have increased risk of several cardiometabolic diseases and often have obesity [11,48,49]. A meta-analysis of research evaluating the effects of obesity on PCOS showed that there was an insignificantly higher risk for impaired glucose and T2DM among women with overweight; however, women with obesity were significantly more likely to experience these metabolic changes associated with PCOS [49]. The reproductive differences (decreased sex hormone-binding globulin, increased total testosterone, free androgen index) excluding hirsutism are all exacerbated by obesity, whereas those women with overweight are not as affected [49].

Although the effects of lifestyle modification (discussed later in this chapter) on reproductive health are uncertain, lifestyle modification does improve cardiometabolic profile (fasting blood glucose, fasting blood insulin, decreased BMI) in women with PCOS [48,50]. There is some literature which points to improved reproductive outcomes with lifestyle modification for weight loss in the woman with obesity and PCOS [50−52]. The relationship between obesity, PCOS, and reproductive outcomes are important as the woman of reproductive age planning to conceive represents an opportunity for the HCP to positively affect two generations.

C Assessment of Readiness and Goal Setting

The weight loss process is physically, mentally, and emotionally demanding for most. In order to appropriately advocate for, educate, and treat the patient with

overweight or obesity HCPs must be able to assess the patients' stage of change and utilize motivational interviewing (MI) techniques to then evoke change within the patient.

1 The Transtheoretical Model of Behavior Change

According to Prochaska et al. [53], "any activity that you initiate to help modify your thinking, feelings or behavior is a change process" (p. 25). The transtheoretical model (TTM) of behavior change was initially utilized in psychotherapy to treat addictive behaviors by treating behavior change in a nonlinear, dynamic fashion [54]. The stages of change include precontemplation (no intention of changing), contemplation (thinking about change within the next 6 months), preparation (beginning to consider change more strongly, perhaps in the next 30 days), action (implementation of the behavior has occurred for the past 30 days), maintenance (behavior change has occurred for 6 months or longer), and termination [53] (Table 22.3). There are some that believe termination never occurs as the patient will always think about and/or struggle to be consistent with their new behaviors and thus, maintenance is often considered the final stage. Regardless, the patient attempting to change various behaviors in an effort to lose weight will "spiral" through these stages, and it is our job as HCPs to recognize where they are in this process in an effort to guide them into the next stage.

2 Motivational Interviewing

Obesity, like the cardiometabolic diseases associated, is a health condition amenable to the effects of healthy diet and regular exercise [55]. Discussing weight and need for weight loss with patients is difficult. Understanding obesity as a disease can potentially make this a less threatening topic for the HCP. MI, although time consuming, can be a useful skill to have when attempting to evoke change in patients, especially considering the intensity of change needed for weight loss and maintenance. It allows for constructive communication regarding the reduction of health risks and changing behavior. When talking with patients about their obesity, it should be discussed as a disease and related to any risk for or presence of comorbid conditions. MI allows the HCP to meet the patient where she or he is by identifying and discussing the difference between their current behaviors and their desired goals. When utilizing this form of communication with patients you want to discuss those discrepancies such that the patient begins to talk about the changes they want and need to make. This is done by establishing rapport with the patient and utilizing open-ended questions, and affirmation, reflection, and summarization of what the patient is saying [56].

TABLE 22.3 Stage of Change

Matching Interventions with Stage of Change	
Precontemplations	Provide knowledge, discuss pros and cons of change
Contemplation	There may be a balance between pros and cons, utilize MI
Preparation	Pros begin to outweigh the cons. Continue utilizing MI techniques.
Action	The pros outweigh the cons allowing the patient to actively begin to change.
Maintenance	Patient continues new behavior. Consistent work to avoid relapse.

Source: Adapted from J.M. Barrow, Promoting health-behavior changes patients, Am. Nurse Today 8 (2013) 43–45.

3 Goal Setting and Clinically Meaningful Weight Loss

Understanding the TTM and utilizing MI skills gives the HCP a great advantage in coming to the aid of patients with obesity who are ready to make the changes necessary to promote weight loss. When caring for the patient with obesity, goal setting can be difficult. Many times patients are either unrealistic about the amount of weight they should lose, or often times the amount of time it should take to achieve such goals. Regardless, goal setting is imperative given it will direct your care plan. For example, a patient who wants to lose over 50% of their excess weight may be best served by referral to a surgical weight loss process. However, a patient determined to lose only 10% of their excess weight will likely be successful with lifestyle modification with or without the additional of antiobesity medications.

As the HCP and patient discuss weight loss goals, it is important to recognize and discuss the benefits of modest weight loss. Research has consistently revealed the clinical significance of modest weight loss of 5–10%. In fact, as little as 2% weight loss has shown to improve hemoglobinA1c (HgbA1c) [57,58]. Increasing weight loss from 2% to 5–10% can delay the progression from prediabetes (PreDM) to T2DM by 50% [58,59]. Decreases in SBP and DBP [58,60], total cholesterol and triglycerides, as well as increased high-density lipoprotein (HDLs) have also been noted with a modest weight loss of 5–10% [58,61].

Not only are there improvements in cardiometabolic risk factors and disease with modest weight loss, there are also improvements in biomechanical ailment and mental illness associated with obesity. The patient with obesity

may experience lower back pain, lower extremity arthralgia, gastroesophageal reflux disease, and mood disorders [62], symptoms which may improve with clinically significant and modest weight loss of 5−10%.

Once the appropriate stage of change is identified and the patient reports increasing motivation, a goal of 5−10% weight loss should be set. Additionally, lifestyle modification goals should be included. Goals should be SMART—specific, measurable, achievable, relevant/realistic, and time-based.

III SELECTING TREATMENT OPTIONS

According to the guidelines set forth by the AHA/ACC/TOS, obesity treatment should be based on patient BMI and cardiometabolic risk [6] (Table 22.4). As the BMI and cardiometabolic profile of the patient increases, the intensity and invasiveness of the treatment also increases. Generally, each patient should begin with lifestyle change, ideally for 6 months prior to initiation of pharmacotherapy [6] with antiobesity medications. For those who have tried and failed lifestyle modification, FDA-approved antiobesity medications should be initiated. If a patient has tried and failed lifestyle modification as well as pharmacotherapy, surgery and some devices are viable interventions.

A Lifestyle Modification

Lifestyle modification requires changes in two aspects of daily life. Patients need to modify their diet and increase physical activity/exercise. Over the past 10 years, there have been two important studies on the effects of lifestyle management in the obese population, specifically related to T2DM.

The Diabetes Prevention Program (DPP) lifestyle program, aimed at a 7% weight loss and150 minutes of physical activity weekly, effectively reduced the incidence of T2DM by 58% [63]. This was a culturally sensitive individually delivered program, involving frequent contact between the 1079 participants and coaches/case managers. The program included a 16 session core curriculum targeting diet, activity, and behavioral self-management topics, supervised physical activity sessions, and follow up weight loss maintenance interventions, with access to local and national networks [63]. The DPP shows us the level of intensity needed to achieve and maintain clinically meaningful weight loss, at least in the PreDM population.

The Look AHEAD (Action for Health in Diabetes) was another randomized controlled clinical trial evaluating the effectiveness of intensive lifestyle interventions (ILIs). This study evaluated people with obesity and T2DM and examined the effects of weight loss and increased activity on cardiovascular morbidity and mortality [64]. Approximately 5000 patients were randomized to either the ILI or a diabetes support and education program. The ILI was a three-phase group and individual intervention. Phase I comprised of two 6-month periods (1 year total), Phase II extended over 2 years (years 2−4), and Phase III began at year 5 and extended to the end of the trial. Sessions were initially delivered weekly in a pattern of three group sessions and one individual session per month for the first 6 months; then two group sessions and one individual session per month for the second 6 months; and then monthly individual sessions with 4- to 6-week refresher groups and campaigns two to three times per year for the remainder of the intervention. Participants received calorie targets based on initial weight and a physical activity goal of 175 minutes weekly and 10,000 steps daily [64]. Those randomized to the lifestyle intervention were able to achieve clinically meaningful weight loss ($>5\%$) within the first year (68%) [65] and maintain that weight loss through 8 years (50%) [66]. The ILI utilized in the Look AHEAD trial demonstrated that long-term weight loss is achievable with ongoing follow up and monitoring [66] and that weight loss is more likely to be maintained if achieved within the first 2 months of the weight loss process [67].

1 Dietary Modification

In order for patients to lose weight, a caloric (energy) deficit must occur. An energy deficit can be created through

TABLE 22.4 Criteria for Treatment of Obesity Based on Guidelines (Based on BMI and Comorbid Conditions)

BMI	25−26.9 kg/m^2	27−29.9 kg/m^2	30−34.9 kg/m^2	35−40 kg/m^2	>40 kg/m^2
Diet, exercise, behavior therapy	X	X	X	X	X
Antiobesity medication		X (in the presence of comorbidity)	X	X	X
Surgery				X (in the presence of comorbidity)	X

diet alone, exercise alone, or a combination of the two. For many, creating energy deficit through caloric restriction is the first step taken. Dietary energy deficits can be created for most women, by decreasing the daily caloric intake to 1200−1500 kcal/day; and for men, with a daily caloric intake of 1500−1800 kcal/day. Reducing a patient's daily intake by 500 kcal/day will create a deficit of 3500 kcal/week, leading to approximately 1 pound of weight loss per week. In order for this particular approach to be successful, one must first estimate the patient's current caloric intake or weight maintenance caloric requirement [6].

a Ad Libitum

For those patients who find structuring their daily intake to allow for accurate calorie counting tedious and time consuming, an ad libitum diet may be more realistic [6]. The ad libitum diet achieves caloric deficit through (1) decreasing portion size, (2) decreasing or eliminating a certain macronutrient like carbohydrates, (3) decreasing or eliminating high caloric, nonnutritional foods like potato chips and candy, or (4) decreasing or eliminating consumption of calorie-rich beverages, such as soda and juices.

b Very Low Calorie Diets and Low Calorie Diets

Very low calorie diets (VLCDs) restrict daily caloric intake to $<$800 kcal/day, whereas LCDs restrict daily caloric intake to $<$1200−1500 kcal/day. Protein shakes and other meal replacement (MR) smoothies or bars are often used to achieve the VLCD caloric requirements.

Given the extremely low daily caloric intake associated with the VLCD, this diet requires almost a full liquid approach. Patients are often on 3−5 shakes daily, with multivitamin and mineral supplementation. Side effects include fatigue, hair loss, dizziness, and constipation; and risk for cholelithiasis secondary to rapid weight loss. The VLCD usually results in $>$20% weight loss within the first 3−4 months [68]. Although rapid weight loss is seen, it is not usually well maintained with many patients gaining up to 50% of that weight back within the subsequent 12 months; and gaining all of the weight back in less than 5 years [69]. LCDs are not as extreme and with almost twice as many calories allowed (1200−1500 kcal/day), the weight lost is modest.

c Protein Sparing Modified Fast

The protein sparing modified fast (PSMF) diet is a 12 to 16 week very low calorie ketogenic treatment diet. Patients are advised to consume high bioavailable protein ideally from animal sources, specifically 1.5 g of protein per kilogram of ideal body weight, based on BMI of 22 kg/m^2 for women and 23 kg/m^2 for men. The PSMF also eliminates all carbohydrates and extra fats. Due to the restrictive nature of this VLCD, the PSMF also requires patients take a multivitamin and supplementation with sodium chloride, potassium, magnesium, and calcium [70] as needed. HCPs can confidently inform their patients that expected weight loss ranges from 1 to 3 kg weekly [70]. When combined with an intensive behavior program, 50% excess weight loss can occur [71]. Based on the findings of Palgi et al. [71] it is reasonable to utilize this diet in the treatment of patients with obesity, especially those that would benefit from significant weight loss but would prefer to avoid surgical options.

d Meal Replacements

MR diets allow the patient to replace one to two meals daily with either a shake (usually high protein) or protein bar. Ideally these MRs are fortified with vitamins and minerals to replace what would be consumed in normal meals. According to Heymsfield et al. [72], the partial meal replacement (PMR) diet can be more effective than reduced calorie diets (RCD) for weight loss. Those adhering to a PMR diet lost 7−8% of their body weight compared to those on the RCD (3−7%). The weight lost by those on a PMR diet was significant at both 3 months and 1 year [72]. This diet may be most effective for patients who (1) are not interested in calorie counting, (2) have difficulty restricting their favorite foods and therefore don't do well on ad lib diets, and/or (3) have hectic schedules which don't allow for food preparation and require eating on the go.

e Macronutrient Composition

Macronutrient diets determine caloric intake based on the percentage or total grams of protein, carbohydrates, and fat intake. Such diets are usually referred to as low-fat/high-carbohydrate diets, low-carbohydrate/high-protein diets, or moderate fat diets. These diets may range from 15% to 25% protein, 20% to 40% fat, and 35% and 65% carbohydrates.

Countless studies have been executed to identify which diet is best. Sacks et al. [73] looked at the results of various macronutrient compositions over the course of 2 years. During this time research looked at the effects of low versus high percentages of each macronutrient on weight and health outcomes. Within the first 6 months participants in all diet groups had clinically significant weight loss regardless of diet composition with weight regain over the next 2 years [73]. When comparing commercial diets, it has been noted that modest weight loss and improvements in cardiometabolic profile can be expected [74].

Research on the best diet for weight loss and the best diet for reducing cardiometabolic morbidity and mortality

is ongoing. What we know at this point is that (1) most calorie restrictive diets will produce weight loss within the first 6 months after which some weight regain will occur, (2) improvements in cardiometabolic profile are also likely to accompany weight loss, and (3) adherence to the diet of choice markedly improves the aforementioned results. Adherence may not only improve initial results, but it may in fact be the factor which increases the possibility of long-term success [74,75].

2 Physical Activity Modification

Dietary interventions alone can account for significant weight loss but results can be amplified and maintained with physical activity and exercise. The American College of Sports Medicine (ACSM) provides recommendations for physical activity based on desired goals. According to ACSM 150 minutes/week of physical activity will maintain or improve health. Initial weight loss is achieved with 150−250 minutes/week, whereas weight maintenance requires almost twice as much exercise (200−300 minutes/week) [76,77]. These recommendations include moderate-to-vigorous physical activity expending 1200−2000 kcal/week [78] (240−500 kcal/day for 5 days/week).

As important as it is for HCP to recognize the value of recommending physical activity, it is equally important that we are able to discuss the detrimental effects of physical inactivity with our patients. Physical inactivity (or sedentary behaviors) are associated with several negative health outcomes including increased risk for T2DM and CVD as well as premature mortality [79−81]. Creating a comprehensive treatment plan for the patient with obesity that discusses physical activity and exercise is imperative, given its known health benefits and potential effect on long-term weight management. When making recommendations to patients, it is important to understand the difference between physical activity and exercise.

3 Physical Activity

Physical activity can be defined as any bodily movement produced by skeletal muscle, ultimately leading to energy expenditure. It differs from exercise as it lacks structure and is typically not associated with fitness goals [82,83]. Examples of physical activity include activities of daily living, yard work, house cleaning, walking for transportation, and taking the stairs instead of the elevator.

We now know that physical inactivity is detrimental to health whereas physical activity improves health. Also, it is widely accepted that physical activity is important in improving the majority of risk factors associated with CVD, such as T2DM, regardless of age. Lifestyle interventions which include a calorie restricted diet, exercise, and weight loss show >50% reduction in developing T2DM [84,85]. Thus, regardless of overall fitness goals, the patient with overweight and obesity should be instructed to increase their overall physical activity and perhaps work toward the well-known recommendation of 10,000 steps/day.

4 Exercise

Physical activity is important, but exercise has significant contributions to overall health as well. Exercise is defined by ACSM as physical activity involving repetitive bodily movements that is planned or structured, with the goal of improving physical fitness (cardio, strength, flexibility, etc.). It includes aerobic training, often referred to as cardiovascular or cardio for short; resistance training (RT), often referred to as strength training; and flexibility training (FT), often referred to as stretching. It is not just enough to exercise, but one must consider the frequency, intensity, time (duration), and type of exercise while working toward goals.

Frequency refers to the number of days per week. As most know, both the CDC and ACSM recommend physical activity/exercise on most days of the week, or 5 days. Intensity refers to the level of difficulty. In much of the research, intensity effort is defined as the percentage of maximal volume of oxygen consumed ($VO2_{max}$), percentage of heart rate, or rate of perceived exertion (RPE). Intensity may be the confounding factor in translating exercise research to patient care and practice. Intensity for many will not be determined by their heart rate or $VO2_{max}$, but by utilizing tools such as the talk test and RPE (Table 22.5). Time refers to how much time in minutes is spent exercising. Lastly type includes aerobic, resistance, and flexibility exercises; and should always be considered fun.

a Aerobic Training (or Endurance Training)

The ACSM defines moderate intensity physical activity as 40−60% and vigorous physical activity as 60−90% of maximal heart rate or heart rate reserve. Moderate aerobic/cardiovascular training should be completed 5 or more days/week, 30−60 minutes/day (150−300 minutes/week),

TABLE 22.5 Comparison of Exercise Intensity, Talk Test, and RPE

Intensity	Talk Test	RPE
Very light	You can talk and/or sing	<3
Light		3−4
Moderate	You can talk, but NOT sing	5−6
Vigorous/hard	Difficult to talk or CANNOT talk	7−9
Very hard max		>10

whereas vigorous (>3 days/week; 20–60 minutes/day or 75–100 minutes/week) and moderate-to-vigorous (3–5 days/week; 20–60 minutes/day or 75–300 minutes/week) aerobic training can be done at a lower volume. Examples of aerobic/cardiovascular training include dancing, running/jogging, swimming, jumping rope; or playing sports like soccer or basketball [82,83].

As HCPs we should confidently tell our patients that improving their cardiorespiratory fitness (CRF) levels through aerobic activity will improve their overall health, especially for men with obesity [86] by decreasing mortality risk.

b Resistance Training

Resistance/strength training should occur at least 2–3 days/week, focusing on each major muscle group. Each muscle group should be stressed over 8–20 repetitions, 1–4 times (sets) with 1–3 minutes of recovery (rest) between sets. In general RT should be progressive, meaning there is some increase in the aforementioned variables with improvements over time [82,83,87].

Some research has noted that the greatest effect of RT on weight loss and conservation of fat-free mass (FFM) occurs with progressive, periodized programs which should thus be recommended more often to the patient with obesity. One practical limitation is that HCPs without the knowledge of exercise prescription/programming do not know how to progress RT routines for individual patients. Perhaps recommendations and guidance on exercise can occur seamlessly when HCP and exercise specialists through ACSM, strength coaches through the National Strength and Conditioning Association (NSCA) and certified personal trainers through various organizations collaborate to create an appropriate periodized program for their patients with overweight and obesity.

c Flexibility Training

Unlike aerobic and RT, FT does not have the same structured guidelines. If flexibility is an issue, daily stretching is considered most effective; otherwise each muscle group should be stretched for 2–3 days/week for at least 10–60 seconds, for 2–4 sets. Stretches can be static (held in place) or dynamic (moving) and should never cause pain [82,83].

5 Exercise and Weight Loss (With or Without Diet)

Physical activity and exercise have been touted as ineffective as a monotherapy for the treatment of obesity. Weight loss through exercise may only yield 0.1 kg/week [88]. Although the initial weight loss may be insignificant clinically, it is important to recognize the benefits of various forms of exercise on body mass, body composition, and cardiometabolic profile.

Clark [87] reviewed 66 studies, and pooled them to compare four specific subgroups including diet-only (D) interventions, combination endurance training (ET) and diet (ET/D), combined RT and diet (RT/D), and the combination of diet, RT, and ET (ET/RT/D). This meta-analysis and systemic review found ET/D versus D, to be more effective at decreasing body mass whereas RT/D was found to be more effective at preserving FFM while decreasing fat mass when compared to D. RT was favored over ET as a means of conserving FFM, alone or in combination with diet. Gender differences have also been noted in response to exercise, diet, and combination of exercise and diet. Women appear to respond to diet alone or in combination with ET, although this is only seen as a reduction in body mass and not conservation of FFM, whereas men tend to respond more so to diet with RT alone or a combination of ET/RT/D.

Although exercise alone may yield insignificant weight loss, we do know exercise is quite effective in weight maintenance or prevention of weight gain [89], even more so than just relying on diet to maintain weight [90].

6 Exercise and Cardiometabolic Improvements

Research repeatedly shows that diet and exercise improve the cardiometabolic profile of the patient [87]. In a study designed to evaluate the effects of physical activity on WC and CHD risk, physical activity, defined as activity at work and during leisure time, was shown to decrease WC independent of change in BMI. Inactive men with elevated WC had a HR for future CHD of 1.74 (95% CI); and women had a HR of 4.0 (95% CI) [91]. Following acute coronary events, cardiac rehabilitation and exercise training also reduce the prevalence of metabolic syndrome by 37% [92]. Despite research suggesting exercise-related improvements in cardiometabolic profile following a cardiac event, patients with obesity may not receive appropriate guidance, as clinicians ponder over which form of exercise should be promoted, RT, ET, or both.

a Resistance Training

RT with or without diet has been shown to improve total cholesterol and low-density lipoprotein (LDL) compared to ET which has shown no significant changes in lipid profile. Both RT and ET, with or without diet, have been shown to improve HDL. The only gender difference seen was related to improvements in HDL, which were in women engaged in ET and diet [87]. RT is noted to decrease the incidence of metabolic syndrome in those who practice, but research is confounding. A meta-analysis of 19 research articles evaluating RT only and its effects on metabolic syndrome, found RT significantly improves BP, fasting plasma glucose, HDL, triglycerides, and WC [93].

b Endurance Training

ET improves CRF. Zaccardi et al. [94] notes an inverse relationship between CRF and incidence of T2DM such that improvements in fitness decrease risk for T2DM by 5% per metabolic equivalent, but there was a wide range of variability in the percentage of T2DM reduction. These variations could be related to the diversity of the study population, but does not negate the positive effects of CRF on T2DM [94].

HCPs understand the value of diet, exercise, and physical activity not only in promoting weight loss but also in improving cardiometabolic profile. Although there is some confusion over what form of exercise to suggest, it is best to recommend a well-rounded, progressive program to our patients. Lifestyle changes incorporating both RT and ET in addition to calorie restriction can invoke the clinically meaningful weight loss associated with cardiometabolic improvements. Lifestyle modification, with behavior modification (BMOD), will serve as the backbone to any comprehensive program chosen for the patient with chronic obesity.

B Behavior Modification

Changing one's behavior is difficult. We know this from the extensive literature on weight loss and smoking cessation. The HCP can assess each patient's stage of change and identify change talk to assist the patient in recognizing where they are in their weight loss process; helping each patient with obesity transition through the stage of change process is vital to their success.

Considering patients with obesity are being advised to begin regular exercise programs, change their diet, perhaps start an antiobesity agent, and other behaviors (i.e., sleep hygiene), it is important to recognize that the stage of change approach may be different for each behavior change recommended. Marshal and Biddle [54] theorized that self-efficacy for change, decisional balance of perceived advantages and disadvantages of change, and the targets and techniques (the process of change) individuals use to modify their thoughts, feelings, and behavior are the main mediating factors in the change process. BMOD skills are likely best taught by a trained psychologist, psychiatrist, therapist, counselor, licensed social worker, or dietitian.

Thus, it is important that the HCP understand that diet, exercise, and behavior change are important parts of a comprehensive program which can illicit up to 10% weight loss and improve or prevent the cardiometabolic cormorbidities associated with obesity [95].

IV PHARMACEUTICAL INTERVENTIONS

A Background

Unlike comorbid diseases, obesity does not have an extensive list of options for pharmacotherapy; and the recognition of obesity as a disease has only modestly improved this. Antiobesity medications are an adjunct to lifestyle changes and ideally should only be initiated after a 6-month trial of diet and exercise [6]. The oldest medications (i.e., phentermine) have been FDA approved and available for almost 90 years. The first drugs, amphetamines, were used in the 1930s as anorexiants, but had to be reformulated because of addiction potential. Since then, several medications have been utilized, of which phentermine is the most often prescribed to date. In the late 1990s, a lipase inhibitor, orlistat, was FDA approved for weight loss. Since 1990 several medications have been developed for the treatment of obesity, but only four remain on the market, lorcaserin, liraglutide, and two combination medications phentermine/topiramate ER and bupropion/naltrexone [96].

Treating obesity, a chronic metabolic disease with cardiometabolic sequelae, is not like treating HTN or diabetes. First, it is important for the HCP to recognize the limited availability of antiobesity medications FDA approved for long-term management of obesity. Second, given the challenge of maintaining long-term compliance with the lifestyle changes, it is often difficult for patients to maintain the weight initially lost, validating the need for pharmaceutical interventions. Finally, when evaluating the cardiometabolic disease risk and medical history of the patient, often times the HCP's options for antiobesity medications are further limited.

B Antiobesity Medications Approved for Short-Term Use in Weight Loss

1 Phentermine Hcl

Phentermine Hcl is a noradrenergic sympathomimetic derived from amphetamine-based molecules, and the most commonly used anorexic agent by health care professionals (Table 22.6). Phentermine is FDA approved for short-term (3 months) use as a result of short-term studies completed during a time when obesity was not considered a disease, let alone a chronic disease. The exact mechanism of action (MOA) is unknown, but it is thought that Phentermine Hcl releases catecholamines in the hypothalamus [97] thereby suppressing appetite.

The most commonly reported adverse effects (AEs) are dry mouth, constipation, jitteriness, difficulty sleeping, and dizziness. Clinically, AEs such as elevated blood pressure and tachycardia should be evaluated at least monthly. Phentermine Hcl is a schedule IV, category X medication. It should be avoided if pregnant or lactating; women of childbearing age should be counseled on this matter. Phentermine Hcl is contraindicated for those with known hypersensitivity, known monoamine oxidase

TABLE 22.6 Long-Term Pharmacotherapeutic Intervention to Promote Weight Loss for the Treatment of Obesity

Agent	MOA	Cost	One Year Weight Loss (kg)	Common Adverse Effects	FDA Approval
Orlistat	Pancreatic lipase inhibitor causing excretion of ~30% of ingested triglycerides in stool	60 mg, $45120 mg, $207 wholesale	60 mg, −2.5 kg (−1.5 to −3.5) 120 mg, −3.4 kg (−3.2 to −3.6)	Oily spotting, flatus with discharge, fecal urgency, fatty oily stool, increased defecation, fecal incontinence	1999 orlistat2007 OTC
Lorcaserin	Highly selective serotonergic 5-HT2C receptor agonist causing appetite suppression	$240 Wholesale; Now $75	−3.2 kg (−2.7 to −3.8)	Headache, dizziness, fatigue, nausea, dry mouth, cough, and constipation; and in patients with type 2 DM: back pain, cough, and hypoglycemia	June 2012
Phentermine/ Topiramate-ER	Noradrenergic + GABA-receptor activator, kainite/ AMPA glutamate receptor inhibitor causing appetite suppression	$140−195	7.5 mg/ 46 mg − 6.7 kg (−5.9 to −7.5) 15 mg/ 92 mg − 8.9 kg (−8.3 to −9.4)	Paresthesias dizziness, taste alterations, insomnia, constipation, dry mouth, elevation in heart rate, memory or cognitive changes	July 2012
Bupropion/ naltrexone	Inhibitor of dopamine and noradrenaline reuptake + μ-opiate antagonist	$60−70 per month	6.2 kg 48 weeks	Nausea, constipation, headache, vomiting, dizziness trouble sleeping, dry mouth diarrhea	September 2014
Liraglutide	GLP-1 agonist	$1000 per month	6.2 kg 104 weeks	Nausea, vomiting, constipation, diarrhea, headache	December 2014

Source: From S.Z. Yanovski, J.A. Ynovski, Long-term drug treatment for obesity: a systematic and clinical review, JAMA 311 (1) (2014) 74−86. Modified from A.G. Powell, C.M. Apovian, L.J. Aronne, New drug targets for the treatment of obesity, Clin. Pharmacol. Ther. 90 (1) (2011) 40−51.

inhibitors (MAOi) use within 14 days, pregnancy, lactation, history of CVD, atherosclerosis, uncontrolled HTN, hyperthyroidism, and glaucoma.

Phentermine Hcl is dosed in 8−37.5 mg tablets, taken by mouth once daily (QD) 30 minutes prior to breakfast. When taken either continuously or intermittently for 36 weeks, phentermine results in greater weight loss compared to placebo. In fact, weight loss can be up to three times that seen with placebo alone (placebo—4.8 kg, continuous—12.2 kg, intermittent—13.0 kg) [98,99] making this antiobesity medication quite effective at producing significant weight loss in the short-term.

C Medications Approved for Long-Term Use

1 Orlistat (Xenical, Alli)

Orlistat, a pancreatic lipase inhibitor, has been FDA approved since 1959 for long-term weight management, at a dosage of 120 mg, three times daily (or 60 mg three times daily over the counter) taken with a fat-containing meal or 1 hour following the meal. Given orlistat prohibits the absorption of fat from the gut; the HCP should recommend a daily multivitamin containing fat-soluble vitamins.

The most common AEs associated with this medication include oily fecal leakage, fecal urgency, and fatty/ oily stool. There has been some postmarket incidence of liver damage and therefore liver function tests should be evaluated with initiation and dose adjustments [100]. Like phentermine and all other antiobesity agents, Orlistat is a schedule X medication, thus should not be taken while pregnant or breastfeeding. Orlistat is contraindicated for known hypersensitivity, pregnancy, chronic malabsorption syndromes, and cholestasis.

Orlistat was noted to have superior weight loss when combined with a lifestyle change program, compared to a lifestyle program alone. Torgerson et al. [101] conducted a 4-year, double-blind, prospective study with 3305 participants with BMI 30 kg/m^2 and normal (79%) or impaired (21%) glucose tolerance (IGT) randomized to (1) maximal dosage of orlistat as an adjunct to lifestyle changes or (2) lifestyle changes alone. After 4 years the group treated

with orlistat lost significantly more weight (5.8 kg) than the lifestyle alone group (3.8 kg) [101].

Within the group with obesity on orlistat, there was no significant difference in weight loss between those with normal or IGT. In addition to increasing weight loss, Orlistat was effective in decreasing the incidence of T2DM, evidenced by a risk reduction of 37.3%. The addition of Orlistat to lifestyle management increases weight loss compared to lifestyle alone, and more importantly it helps the patient with obesity to maintain clinically significant weight loss long-term (4 years) [101].

2 Phentermine/Topiramate ER (Qsymia)

Phentermine Hcl, already approved for the short-term treatment of obesity, was combined with topiramate, a dopamine/GABA inhibitor traditionally used to treat migraines and seizures, for the long-term (up to 1 year) treatment of obesity, and FDA approved in 2012. Phentermine Hcl/Topiramate ER dosages start at 3.75 mg/23 mg, respectively, and increase incrementally to a maximal dosage of 15 mg/92 mg, taken QD. The MOA of phentermine was discussed previously. The additional effects of topiramate ER involve several potential MOAs which effect GABA activity, AMPA/Kainite excitatory glutamate receptors, and carbonic anhydrase [97]. Combined, both centrally acting agents promote an anorexogenic effect leading to significant weight loss.

The most common AEs are paresthesia, dizziness, cognitive dysfunction, dysgeusia, insomnia, constipation, dry mouth, metabolic acidosis, and elevated creatinine [97]. Phentermine Hcl/topiramate ER is a scheduled IV drug, because of the potential addicting and stimulating effects of phentermine Hcl; and pregnancy category X due to teratogenic effects of topiramate. Topiramate causes cleft palate in the first trimester of pregnancy, thus all women of childbearing age should utilize some form of contraception prior to initiation and throughout continued use of the medication as recommended by the Risk Evaluation and Mitigation Strategy program. Phentermine Hcl/Topiramate ER is contraindicated for use with known hypersensitivity, MAOi use within 14 days, pregnancy, lactation, CVD history, hyperthyroidism, glaucoma, and agitation.

Phase II trials of phentermine Hcl/topiramate ER suggest the antiobesity medication effectively improves glycemic control in patients with obesity and T2DM and improves sleep apnea. EQUATE, a randomized, double-blind, placebo-controlled 28-week phase III trial, illustrated the superiority of monotherapy using phentermine Hcl/topiramate ER over the individual components. EQUIP, a 56-week phase III trial, demonstrated the dose-dependent effect of 1 year phentermine Hcl/topiramate ER use on weight loss (−5.1% 3.75/23 mg, −10.9% 15/92 mg) compared to placebo (−1.6%). More importantly, this trial shows us that those patients who qualify for bariatric surgery have a medical alternative which is also effective [97] as those with BMI >40 kg/m^2 were able to achieve weight loss greater than 10%, and with some greater than 15%.

CONQUER, which was the largest of the phase III randomized control trials, began to examine the effects of phentermine Hcl/topiramate ER on the cardiometabolic diseases associated with obesity. Again, phentermine Hcl/topiramate ER led to greater losses in weight, with many achieving 5% and 10% weight losses. More importantly, the use of phentermine Hcl/topiramate ER led to improvements in HTN, lipid profile, and glycemic control. Improvements were such that patients were able to decrease the use of medications to treat these cardiometabolic comorbidities associated with obesity [97].

The CONQUER trial was extended 52 weeks and became SEQUEL, a 108-week, phase III randomized control trial, which showed that the weight loss and cardiometabolic changes experienced within the first year of phentermine Hcl/topiramate ER use could be sustained through a second year, suggesting the value of long-term use of this antiobesity medications for the treatment of obesity [97].

3 Lorcaserin (Belviq)

Lorcaserin is a serotonin (5HT) agonist, FDA approved in 2012 for long-term (up to 2 years) management of obesity, taken as 10 mg tablets twice daily (BID). Lorcaserin is a 5HT2C-specific agonist, unlike previous nonselective agonists, fenfluramine and dexfenflurame, which activated 5HT2B receptors causing valvulopathy [102]. The MOA is not completely understood; however, it is thought to decrease food intake and increase satiety through the activation of the pro-opiomelanocortin (POMC) system of the hypothalamus.

The most commonly reported AEs are fatigue, constipation, dry mouth, headache, nausea, and dizziness. Due to the risk of serotonin syndrome, concomitant use with other serotonin agonists (i.e., selective serotonin reuptake inhibitors, SSRIs) should be avoided. Given lorcaserin is a 5HT-agonist and previous antiobesity agents with similar mechanisms of action led to valvulopathy, it is important to know that with serial echocardiograms, lorcaserin showed no increased risk of valvulopathy when taken BID [103]. In general this is a well-tolerated and safe antiobesity medication, which elicits clinically meaningful weight loss among patients with overweight and obesity, with or without diabetes [104]. Lorcaserin use should be avoided in those who have known hypersensitivity, during pregnancy or lactation, and in those who have decreased kidney function (creatinine clearance, CrCL <30).

When taking lorcaserin QD or BID compared to placebo, overweight and obese patients are more likely to achieve 5% (40.2%, 47.2%, 25%) and 10% (17.4%, 22.6%, 9.7%) weight loss. Following 1 year of treatment those patients who remain on the medication, even after reaching their nadir weight, experience less weight regain than those who discontinue medication use [103].

The BLOSSOM study was a randomized, placebo-controlled, double-blind parallel arm trial. Participants ($n = 4008$) with overweight and obesity were randomized to lorcaserin 10 mg BID, lorcaserin 10 mg QD or placebo groups. The primary outcome of this study was to evaluate the effectiveness of lorcaserin in promoting clinically significant weight loss compared to placebo. Participants receiving lorcaserin were significantly more likely to achieve 5% (47.2% BID, 40.2% QD) and 10% (22.6% BID, 17.4% QD) weight loss [103].

The BLOOM study was a 52-week, double-blind randomized control trial. During BLOOM, 3182 persons with overweight or obesity received lorcaserin 10 mg or placebo, BID. After 52 weeks those taking lorcaserin ($n = 1595$) either stayed on lorcaserin or were reassigned to placebo. Those on placebo ($n = 1587$) initially remained on placebo. At the end of the first year, those on lorcaserin were twice as likely to achieve clinically significant weight loss of 5% and three times likely to achieve 10% weight loss, compared to placebo. The weight loss achieved during the first year was maintained in those receiving lorcaserin during the second year. Whereas those who discontinued lorcaserin in the second year had weight regain.

Similarly to previously discussed antiobesity agents, lorcaserin improved the WC and cardiometabolic profile (fasting glucose, insulin, glycated hemoglobin levels, total cholesterol, LDL, triglycerides, high-sensitive c-reactive protein, fibrinogen levels, SBP, and DBP). Many of the improvements noted in year 1, diminished during year 2 if participants were randomized from treatment to placebo arm [105].

BLOOM-DM was a 52-week, randomized, placebo-controlled clinical trial, evaluating the effects of lorcaserin 10 mg QD, BID, or placebo on weight loss in participants with overweight, obesity, and T2DM. Including modified intent to treat and last observation carried forward data, this clinical trial supports previous findings suggesting taking lorcaserin 10 mg BID compared to QD or placebo, will elicit clinically meaningful weight loss. Unlike previous studies, those taking lorcaserin QD were more likely to achieve 5% (44.7%) and 10% (18.1%) weight loss compared to those on BID dosing (37.5% and 16.3%, respectively). Given this population with overweight and obesity also had T2DM, it is important to recognize the improvements noted in glycemic control for those in each group. As expected, those in the treatment arms were more successful at reducing their HgbA1c, fasting glucose, insulin, and calculated insulin resistance, compared to those in the placebo groups. Greater improvements in glycemic control also resulted in a decrease in use of oral antidiabetic medications [106].

4 Naltrexone Hcl/Bupropion Hcl (Contrave)

Naltrexone Hcl/Bupropion Hcl is a combination of an aminoketone antidepressant, and opioid antagonist provided in a fixed 8 mg/90 mg tablet, taken as 2 tabs, BID for a total 32 mg/360 mg dosage. Bupropion activates "hypothalamic POMC neurons, with downstream effects to reduce food intake and increase energy expenditure" (p. 225). Naltrexone "blocks opioid receptor-mediated POMC de-activation, augmenting POMC firing synergistically" (p. 225). Ultimately, central nervous system (CNS) reward pathways are altered such that the fixed dosage can stimulate weight loss [12].

The most common AEs noted are headache, constipation, dizziness, vomiting, and dry mouth. Although suicidality has not reported any side effect, the HCP should discuss this with patients given the potential mood changes secondary to bupropion use. Naltrexone Hcl/Bupropion Hcl is contraindicated for use with those who have known hypersensitivity, have used MAOi within 14 days, during pregnancy, and lactation, and with end-stage renal disease, uncontrolled HTN, history of seizures or seizure disorder, bulimia or anorexia, acute withdrawal from or dependence of opioids, active alcohol use, or abrupt discontinuation of known CNS depressants (i.e., benzodiazepines).

The Contrave Obesity Research I (COR-I), a double-blind, placebo-controlled, phase III trial, which evaluated the effectiveness of bupropion/naltrexone on weight loss in 1742 overweight and obese patients randomized to hypocaloric diet and exercise in addition to either placebo, NB32 (32 mg naltrexone Hcl/360 mg bupropion Hcl) or NB16 (16 mg naltrexone Hcl/360 mg bupropion Hcl), in BID fixed dose tablets. COR-I demonstrated a dose-dependent weight loss response with the placebo group losing the least amount of weight (−1.3%) compared to NB16 (−5.0%) and NB32 (−6.1%). Similar dose-dependent outcomes were seen in terms of the percent of participants who achieved clinically significant weight loss of 55−84 (16%), 186 (39%), and 226 (48%) for placebo, NB16, and NB32, respectively. Ultimately, COR-I illustrates the effectiveness of NB32 as a treatment option for obesity [107].

The COR-II, a double-blind, placebo-controlled study, expanded on the research done in COR-I to include the evaluation of 1496 patients with overweight or obesity, dyslipidemia, and/or HTN, randomized to either NB32 or placebo groups. Similar to COR-I, there was a statistically

significant difference in weight loss at 28 (6.5%) and 56 weeks (6.4%) in the NB32-treated patients compared to placebo (1.9%, 1.2%, respectively). In addition, there were improvements in WC, lipid profile, insulin sensitivity as well as food cravings, and overall hunger. This study suggests that great improvements in weight and cardiometabolic risk factors can be achieved within 28 weeks of initiating NB32 and be sustained throughout a year [108].

The COR-BMOD, a randomized, placebo-controlled trial, evaluated the efficacy and safety of NB32 plus intensive BMOD in 793 adults with obesity randomized to either NB32/BMOD or placebo/BMOD groups over 56 weeks. Participants were asked to follow a caloric-deficit diet and participate in 28 group BMOD sessions. After 56 weeks the NB32/BMOD group lost 4% more weight than the placebo/BMOD group (9.3%, 5.1%, respectively). When analysis included completers only the NB3/BMOD group lost 11.5% and the placebo/BMOD group lost 7.3% [109]. Additionally there were significant improvements in cardiometabolic disease risk.

COR-DM evaluated the efficacy of NB32 in 505 patients with obesity and T2DM over a 56-week, double-blind, placebo-controlled study, and assessed outcomes of 5% and 10% weight loss, and improvement in HgbA1c, WC, fasting blood glucose, and lipids. All participants received the same lifestyle intervention and were randomized to NB32 or placebo [110]. Those individuals in the treatment arm lost significantly more weight (25.0% NB32; 21.8% placebo). Of those on NB32 approximately 45% were able to lose 5% of their weight, compared to <20% in the placebo group. While on NB32 the participants with overweight or obesity and T2DM were able to reduce their HgbA1c, some to 7%, and improve their lipid profile [110]. Based on these findings, NB32 helps patients with overweight or obesity and T2DM lose weight and improve their cardiometabolic profile.

5 Liraglitide (Saxenda)

Liraglutide 3.0 mg is a glucagon-like peptide (GLP-1) agonist subcutaneous injection, FDA approved in 2015 for use in conjunction with an RCD and regular exercise to promote weight loss according to the AHA/ACA/TOS guidelines. Unlike liraglutide 1.8 mg, the 3.0 mg dosage is not indicated for management of glycemia in patients with T2DM. Liraglutide is an acylated human GLP-1 receptor agonist with >95% similarity to endogenous GLP-1. Glucagon like protein-1 regulates appetite and caloric intake, slows gastric emptying, promotes glucose-mediated insulin secretion, and down regulates glucagon secretion.

The most common AE is nausea, which is why this drug has a dose titration schedule over the course of a month. Patients should be instructed to start with 0.6 mg QD injection for 1 week. Each subsequent week there should be an increase in the dosage as follows: 1.2 mg, 1.8 mg, 2.4 mg, and concluding at the maximal dosage of 3.0 mg daily. Other AEs include vomiting, diarrhea, constipation, hypoglycemia (in patients with diabetes), and headache. Similar to all other antiobesity agents, liraglutide 3.0 mg cannot be taken during pregnancy or lactation. Liraglutide 3.0 mg is contraindicated in those who have experienced medullary thyroid carcinoma (familial or personal), multiple endocrine neoplasia syndrome type 2, or prior hypersensitivity.

SCALE Obesity and Prevention was a 56-week randomized, double-blind, placebo-controlled trial evaluating the effectiveness of Liraglutide 3.0 mg, in addition to diet and exercise in 3731 patients with obesity. Participants in the treatment arm lost almost three times as much weight (~8% liraglutide 3.0, ~3% placebo); and more participants taking this antiobesity agent were able to achieve >5% (~64%) and >10% (~33%) weight loss compared to placebo (~27% and ~11%, respectively). Similar to the results seen in research on other antiobesity agents, this study showed improvements in several cardiometabolic risk factors including WC, glycemia, blood pressure, and lipid levels [111].

SCALE Diabetes was a 56-week international, double-blind, placebo-controlled, parallel-group trial with a 12-week observational off drug follow up period, evaluating the effectiveness of liraglutide 3.0 mg in conjunction with a VLCD (500 kcal/day) and physical activity (>150 minutes/week) in participants with obesity and T2DM. Those receiving liraglutide 3.0 mg daily lost three times as much as those in the placebo group (6.0% vs 2.0%, respectively). Approximately twice as many patients in the treatment arm (~54%) were able to lose 5% of their initial weight compared to the placebo group (~21%); whereas there was almost a fourfold increase in the number of obese individuals able to achieve 10% weight loss on liraglutide 3.0 mg (25%) compared to placebo (~7%) [112].

SCALE Maintenance was a randomized, 56-week trial that evaluated the effectiveness of liraglutide 3.0 mg in maintaining clinically meaningful weight loss (>5%) in obese individuals following a calorically restricted diet. During the initial screening period, approximately 6% weight loss was achieved in all participants. Participants were then randomized to the treatment arm (3.0 mg liraglutide) or placebo for the 56-week study. Liraglutide 3.0 mg increased weight loss by an additional 6% compared to an additional 2% in the placebo group. More participants maintained their initial weight loss while on liraglutide (81%) compared to placebo (49%). Liraglutide 3.0 mg also effectively improved BMI, WC, and glycemic parameters [114].

Given the effectiveness of antiobesity agents in producing short-term weight loss, it is important to continue to work on medications that are capable of promoting

weight maintenance. Astrup et al. [113] evaluated the long-term efficacy of liraglutide 3.0 mg for sustaining weight loss over a 2-year period in a randomized, double-blind, placebo-controlled 20-week study. This European study showed that over the first year those participants on liralgutide 3.0 mg were able to lose almost twice as much weight as those on placebo (5.8 kg, 3.8 kg, respectively); and were able to maintain a 7.8 kg weight loss at 2 years, losing approximately 15.4% body fat. In addition, liraglutide 3.0 mg helps improve cardiometabolic profile (decreased PreDM and metabolic syndrome prevalence, blood pressure, lipid profile) [113].

Since patients often come to their HCP after experiencing multiple cycles of weight loss and subsequent weight regain from trying diet after diet, commonly referred to as "yo-yo" dieting, research to determine the effectiveness of antiobesity medications after initial weight loss with a LCD or intensive lifestyle program would be beneficial. Phase III trials for Liraglutide 3.0 mg have evaluated its efficacy in maintaining 5% weight loss achieved with a LCD. Following initial weight loss, participants with obesity were randomized to liraglutide 3.0 mg per day or placebo for 1 year. Wadden et al. [114] noted liraglutide 3.0 mg as an adjunct to diet and exercise was effective at maintaining initial weight loss seen with LCD. As HCPs and organizations such as TOS work to promote the treatment of obesity as all other chronic diseases, identifying antiobesity agents which improve long-term weight maintenance, such as liraglutide 3.0 mg, opens the door to improved interventions for this population [114].

D Medications Combined With Lifestyle Modification

Guidelines recommend that all patients begin with lifestyle management, including a calorie reducing diet, increased physical activity/dedicated exercise, and some behavior change [6]. Ideally this should begin 6 months prior to the initiation of antiobesity medications. Generally, one can expect about 3–5% weight loss with lifestyle modification alone. Combining antiobesity medications with intensive lifestyle treatment can increase weight loss by an additional 3–8%. Considering the recidivism seen in the weight loss process, having alternative antiobesity medications to combine with lifestyle interventions can only improve the long-term outcomes in the overweight and obese populations.

E Risk: Benefit Ratio

1 Selecting Pharmacotherapy for Patients

When determining the appropriate antiobesity medication for patients with obesity, the health care professional should bear in mind age, comorbid conditions, plans for conception, and initial BMI and weight loss goals of each patient prior to initiating pharmacotherapy.

a. Age: The upper age limit of the research evaluating antiobesity medications is 65 years of age. When prescribing antiobesity agents for your geriatric patients with overweight and obesity, it is important to keep this in mind. This does not mean that our older adult population should be ignored, but it may require more "finesse," and the HCP may want to "start low and go slow" when creating a comprehensive program which includes pharmacotherapy. The pediatric population, although affected by obesity, are not represented in research on anti-obesity medications. Although, not yet discussed, few antiobesity medications are FDA approved for the treatment of overweight and obesity in the pediatric population.

b. Comorbid conditions: Although diet, exercise, and weight loss are often principle concepts in the treatment of many of the health conditions associated with obesity, there are some details which should be noted when considering antiobesity medications for certain populations.

1. T2DM is not a contraindication to the use of any antiobesity medications. What must be considered is the potential for hypoglycemia. Thus, it may be necessary for patients to begin checking their blood sugars more often than they are accustomed, initially or throughout the weight loss process. This is especially important for those patients taking insulin or sulfonylureas.
2. HTN is not a contraindication, unless uncontrolled. For those patients interested in starting an antiobesity medication, controlling their HTN is essential prior to initiating treatment. Given that phentermine Hcl and phentermine Hcl/topiramate ER combination potentially increases BP, the HCP may want to avoid use of this medication in a patient with difficult to control HTN.
3. CVD is a contraindication for the use of phentermine and phentermine/topiramate ER.

c. Plans for conception and/or PCOS: Women of childbearing age and obesity are often counseled to lose weight to improve their chances of conception and outcomes during and after pregnancy. In this population avoiding the use of phentermine/topiramate ER would be advised; however, the use of other antiobesity medication options should be discussed with these patients. All antiobesity medications are contraindicated during pregnancy and lactation.

d. Initial BMI and weight loss goals: Often times patients will ask what their "ideal body weight" is. What we know is that there is increased risk of morbidity and

mortality with extremely low and extremely high BMI. Generally, a BMI of 22 kg/m^2 for a woman and 23 kg/m^2 for a male is considered "ideal" clinically. As a HCP one must be conscious of the patients' initial weight prior to determining and recommending achieving a particular BMI or weight. For example, a patient with a BMI >45 kg/m^2 may never achieve an "ideal" body weight, but may have a goal weight of 100-pounds less than his/her current weight. This is the time when patients need the most guidance and counseling around the medical and surgical options available to treat their obesity. Most importantly, this may be a time to address stages of change and barriers to weight loss, utilize MI, and set SMART goals. In general patients with a BMI >40 kg/m^2 may benefit most from a combination drug such as phentermine/topiramate ER as this drug was shown to successfully lead to 10−20% weight loss at maximal dosages.

V WEIGHT MAINTENANCE

What we really want for patients with obesity is not just weight loss, but weight loss maintenance. We know from countless studies that weight loss is in fact achievable within the first 6 months, but weight regain, ~30%, is likely to occur by the first year, especially when patients are lost to follow-up [115]. What may be the most discouraging fact for patients with overweight and obesity is that many will regain all of their weight, if not more within 3−5 years [116,117]. Thus weight maintenance is in fact the most difficult part of the treatment paradigm for the HCP.

Perhaps, the lower caloric needs following weight loss contribute to the weight regain seen in patients with obesity trying to successfully maintain their weight loss. We know that weight loss causes a decrease in FFM and a decrease in resting energy expenditure (REE). Schwartz and Doucet [118] reviewed 99 papers, from 1969 to 2008, totaling 2996 subjects to evaluate the effects of weight loss on REE and weight regain. This systematic review revealed that the decrease in REE was ~15 kcal/kg of weight lost, independent of intervention or gender and was not correlated to the amount of FFM lost. What was suggested was that exercise in addition to diet may minimize the changes in REE [118]. Although the data from the work of [118] is not comprehensive in explaining the biological basis of weight regain, it is beneficial to understand, but should not distract from the reality and challenges of weight loss maintenance.

The Look AHEAD study implemented the "longest, continuously implemented lifestyle intervention for weight management" [119]. Look AHEAD study identified several key factors related to successful weight loss maintenance. The greater the weight loss in the first year, the greater the long-term weight loss; those who lost 10% of their weight in the first year were 10 times more likely to achieve 10% weight loss in 4 years; and more likely to sustain a 5% weight loss [119].

If the Look AHEAD research gives us weight loss targets over the first year, there is still the question of how to coach patients to success. Given the prevalence of overweight and obesity, there are a great number of people in need of treatment and our most effective intervention is frequent contact [120−122]. Consistently seeing patients keeps them engaged and on track. Helping patients remain engaged by counseling them to record their dietary intake [123] and remain on a LCD [124]; promoting regular self-monitoring of weight [121,125]; and fostering engagement in consistent progressive physical activity/exercise [115−127] are important in aiding patients to adopt and successfully sustain a new lifestyle.

Can we create *Successful Losers*? The National Weight Control Registry, which has tracked over 10,000 people who have maintained a 30-pound weight loss over a year or longer [128] has shown that individuals who commit to 60 minutes of daily physical activity, maintain a low-calorie/low-fat diet weekdays and weekends, regularly have breakfast and monitor their weight [129] and have a greater chance of longer term success with weight loss. It is possible that after practicing these behaviors over time (2−5 years), individuals may have a greater chance at long-term success. Since we know that the faster and greater the weight loss in the first year increases the chances of weight loss at 4 years [119], HCPs can inform patients that they have a greater chance of long-term weight loss [129] when they start and adhere to a plan for the first year. Those with overweight and obesity have greater chances of weight loss and weight maintenance with the help of HCPs who are properly informed on the evidence-based interventions that can maximize initial weight loss and then sustain long-term weight loss maintenance.

VI PEDIATRIC AND ADOLESCENT OBESITY

Adults are not the only segment of the population affected by overweight and obesity. From 1980 to 2012, the prevalence of childhood obesity in the United States has more than doubled (from 7% to 18%) and it has more than quadrupled among adolescents (from 5% to 21%) [2]. Data from the 2013 Youth Risk Behavior Surveillance System (YRBSS) shows that over 13% of adolescents are overweight or obese (CCD, 2014). Children and teens with obesity are more likely to carry their excess weight into adulthood and increase their risk of the same comorbid conditions which affect adults with obesity [1]. Not only

are these children's health affected by excess weight, but they are also more likely to endure the negativity of social stigma and lower academic [1] performance. Given the potential for lower self-esteem, poor academic performance, social ridicule, and long-term health consequences, it is important that the HCP working with the pediatric population with overweight and obesity be prepared.

Childhood and adolescent obesity, like adult obesity, is pandemic and like the treatment paradigm of adults, our younger obese population's lifestyle modification should occur prior to initiation of pharmacotherapy or consideration of surgery [130]. The American Academy of Pediatrics recommends a stepwise care approach including 4 steps: prevention, structured weight management, comprehensive multidisciplinary intervention, and tertiary care intervention [131]. Despite limitations in the research, Spear et al. [131] suggest that lifestyle modification is warranted in this population and should include various diets, address food behaviors, physical activity, sedentary time, and utilize behavior change techniques. These recommendations do not differ from those made for adults. Pharmacological interventions should be left to the HCP in tertiary/specialty care clinics. Of the antiobesity agents once used for the treatment of obesity, orlistat (children >12 years old) and sibutramine were the only FDA-approved medications for use in the pediatric population [131]; however, the sibutramine has been discontinued from the U.S. market due to a cardiovascular incident.

Throughout the lifespan, morbid/severe obesity is difficult to treat, such that bariatric surgery is considered a better option for these patients. Despite the limitation in evidence supporting long-term outcomes, bariatric surgery should be reserved for the morbidly obese adolescent [130].

VII THE FUTURE OF OBESITY

As health care practitioners, our goal of decreasing morbidity and mortality is ignored when we ignore the weight of our patients and consider the treatment of obesity as a cosmetic disease only necessitating diet and exercise. We know that obesity is associated with higher rates of mortality than overweight or normal weight conditions [4]. Decreasing overall excess weight should be a priority. As research tells us that a BMI between 22 and 25 kg/m^2 is ideal, and an increase in BMI of 5 kg/m^2 is associated with a 30% increase in all-cause mortality (in nonsmokers) [4].

Fighting obesity requires that we abandon single-minded approaches and "understand that decisions are not made in a vacuum" ([1], p. 5). One must understand and accept that those communities greatly affected by obesity often lack access to nutritious foods or safe areas for physical activity and need more than just the help of the HCP. Efforts and collaborations among "the individual, family, schools, communities, businesses, government and obesity organizations" are needed ([1], p. 5).

Governmental changes have occurred over the past 5 years to improve the outreach to children and families affected by obesity. There has been an increase in nutrition education and support through the Special Supplemental Nutrition Assistance Program (SNAP-Ed), Supplemental Nutrition Program for Women, Infants, and Children (WIC), and the Child Care Development Block Grant (CCDBG) which offer nutritious food choices and promote physical activity within the communities served. One of the most proliferative governmental programs belongs to former First Lady, Michelle Obama. The Let's Move! program has enlisted the help of key stakeholders in the fight against obesity all across the United States [1] and under the Affordable Care Act, the focus on preventative health care has increased efforts to treat those with obesity.

International organizations such as the International Obesity Task Force (IOTF), TOS, the Obesity Action Coalition (OAC), and the World Obesity Federation (WOF), are dedicated to research and advocacy in obesity. Ultimately, the goal of all stakeholders should be to reduce the prevalence of obesity through prevention and treatment.

VIII CONCLUSION

Obesity is a pandemic, noncommunicable chronic disease, affecting all races, socioeconomic classes, and genders and this chapter only touches the surface of what is Obesity Medicine. This is meant to be an overview. We have come a long way, such that the AMA now recognizes obesity as a chronic disease with health consequences, thereby justifying assessment and treatment of this disease; but there is still much more work to be done.

After reading this chapter you as the HCP should understand the necessity of assessing for obesity by measuring weight, BMI, and WC. The degree of severity should be evaluated by identifying comorbidities and utilizing the EOSS as a way to identify those at risk for long-term health and physical consequences. The HCP should confidently motivate patients to pick any diet program that they can adapt to and be consistent with for life, for we now know adherence to lifestyle change is key [132]. Although the HCP may not be completely abreast of the latest exercise-related research, the basic exercise recommendations from ACSM are a good start. One should be able to assess each patient's stage of change and recognize the role of MI in communicating with and motivating patients to successfully incorporate the behavior changes that will help them accomplish their weight loss goals.

Utilizing the guidelines set forth by the Endocrine Society will allow the HCP educate and coach patients to make lifestyle changes related to diet and exercise. The results of the DPP and Look AHEAD ILIs provide guidance for what our patients should experience from a lifestyle/behavioral perspective. Finally, remember that there are pharmaceutical interventions effective in treating obesity and improving cardiometabolic comorbid disease.

As a nation, HCP, parents, educators, researchers, politicians, faith-based leaders, and the like, we have to understand, accept and implement a multipronged approach to treat and prevent obesity within our communities and rely on the current and up-and-coming research evidence to guide our patients and clinical decisions.

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Chapter 23

Surgery for Severe Obesity

Robert F. Kushner[1,2] **and Holly Herrington**[3]

[1]Northwestern University Feinberg School of Medicine, Chicago, IL, United States, [2]Center for Lifestyle Medicine, Northwestern Medicine, Chicago, IL, United States, [3]Center for Lifestyle Medicine, Northwestern Memorial Group, Chicago, IL, United States

I INTRODUCTION

Bariatric (weight loss) surgery is considered as the most effective treatment for severe (morbid) obesity. According to a survey published by the International Federation for the Surgery of Obesity and Metabolic Diseases (IFSO), over 45,000 bariatric procedures were performed worldwide in 2013 [1]. In the United States, nearly 600,000 bariatric operations were performed over a 5-year period from 2008 to 2012 [2]. The average age of the US patient was 44 years old and approximately three-fourths were female. Surgeries performed include restrictive procedures such as laparoscopic adjustable gastric banding (LAGB), vertical sleeve gastrectomy (VSG), and restrictive-malabsporptive procedures such as the Roux-en-Y gastric bypass (RYGB), and biliopancreatic diversion (BPD and biliopancreatic diversion with duodenal switch, DS). Bariatric surgery is approved as a weight-loss option for two groups of patients: (1) those with a body mass index (BMI) $\geq$40 kg/m^2 (class III obesity) for whom surgery would not be associated with excessive risk, and (2) those with a BMI $\geq$35 kg/m^2 (class II obesity) with one or more severe obesity-related comorbidity, such as diabetes, cardiovascular disease, or obstructive sleep apnea [3–5]. According to the AHA/ACC/TOS 2014 Guideline for the Management of Overweight and Obesity in Adults [5], patients who are motivated to lose weight and who have not responded to behavioral treatment with or without pharmacotherapy with sufficient weight loss to achieve targeted health outcome goals should be advised that bariatric surgery may be an appropriate option to improve health. Furthermore, they should be offered referral to an experienced bariatric surgeon for consultation and evaluation.

Although the number of procedures performed annually has generally plateaued, the prevalence of severe obesity has steadily increased in the United States. Between 1986 and 2000, the prevalence of severe class III obesity (BMI $\geq$40 kg/m^2) quadrupled from approximately 1 in 200 adult Americans to 1 in 50; the prevalence of a BMI $\geq$50 kg/m^2 increased by a factor of 5, from approximately 1 in 2000 to 1 in 400 [6]. The latest available data from NHANES 2011–2012 shows that 6.4% of US adults are severely obese and 8.1% are moderately obese (BMI $\geq$ 35 kg/m^2) [7]. The prevalence of moderate and severe obesity combined is highest for non-Hispanic Black females, which reached 29.3% in the latest survey.

Although the clinical benefits of surgery are well documented, many of the weight-loss surgeries, most notably the combined restrictive–malabsorptive surgical procedures, place patients at high risk for development of both macro- and micronutrient deficiencies unless they are properly counseled and supplemented. Because most of the deficiencies can be identified early at a preclinical stage, early treatment will prevent or reduce symptoms and deficiency syndromes [8]. The role of the Registered Dietitian (RD) in providing medical nutrition therapy (MNT) to these patients is crucial. The dietitian's contribution to the success of the procedure involves practical nutrition knowledge, guidance and encouragement for diet, and behavioral changes. It is important that dietitians provide ongoing medical nutritional treatment, both preoperatively and postoperatively [9].

Although bariatric surgery does not cure obesity, it is considered a significant tool for weight loss and maintenance of weight loss. As such, patients are at risk to experience weight regain several years following surgery. This chapter reviews the most commonly performed weight-loss procedures, the importance of preoperative and postoperative management, the identification and management

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of nutritional deficiencies that may occur following bariatric surgery, and factors associated with weight regain.

II BARIATRIC SURGICAL PROCEDURES

A Mechanisms of Action

Bariatric surgery results in weight loss and improvement of multiple comorbid conditions. Depending on the surgical procedure performed, weight loss primarily occurs by reducing caloric intake, altering gastrointestinal (GI) physiology, and gut–brain neuroendocrine signaling [10]. In contrast to popular perceptions, weight loss does not occur by producing significant macronutrient malabsorption. Instead, research over the past decade has identified multiple functional and hormonal changes involved in hunger, food intake, satiety, and glucose metabolism that occur as a result of the surgical procedures and further explain the differences in resultant weight loss and improvements in comorbid conditions. For this reason, bariatric surgeries are now more aptly referred to as "metabolic procedures." Depending upon the procedure performed, changes in some or all of the gut hormones include reduction in ghrelin, and increase in glucagon-like peptide-1 (GLP-1) and peptide YY_{3-36} resulting in significant changes in hunger, satiety, and food preferences [11,12].

The improvement in comorbid conditions that accompany weight loss is due to multiple interacting factors that effect changes in metabolism, pressure dynamics, and mechanics. Whereas some of the changes are brought about by diet and weight loss alone, others are the result of anatomical and physiological changes of the GI tract. The loss of fat mass, particularly visceral fat, is associated with multiple metabolic, adipokine, and inflammatory changes that include improved insulin sensitivity and glucose disposal, reduced free fatty acid flux, increased adiponectin levels, and decreased interleukin-6, tumor necrosis factor-α, and high-sensitivity C-reactive protein levels. Loss of visceral fat may also reduce intraabdominal pressure that may be related to urinary incontinence, gastroesophageal reflux, and hypoventilation [13]. Metabolic effects resulting from bypassing the foregut include altered responses of gut hormones involved in glucose regulation and appetite control [14].

Fluid and hemodynamic changes related to hypertension include diuresis, naturesis, reduced total body water and blood volume, and decreased indices of sympathetic activity. Mechanical improvements include less weight bearing on joints, improved lung compliance, and reduced bulky fatty tissue around the neck to relieve obstruction to breathing. More recent insights have shed light on altered bile acid metabolism and the role of gut microbiota on improving metabolic health. Depending on the comorbid condition, some or all of these changes may be responsible for improvement or resolution of the comorbidity.

III WEIGHT-LOSS SURGERIES

Weight-loss surgeries have traditionally been classified into three categories: restrictive, restrictive–malabsorptive, and malabsorptive. However, as noted above, newer understanding of the physiological and metabolic mechanisms of action have called this classification into question. Nonetheless, in order to appreciate the nutritional implications and consequences of the surgical procedures, using the traditional classification seems reasonable and will be used here. A diagram of the most commonly performed surgical procedures is shown in Fig. 23.1.

A Restrictive Surgeries

Restrictive procedures limit the amount of food the stomach can hold and slow the rate of gastric emptying. The vertical banded gastroplasty (VBG) is the prototype of this category but is no longer performed due to limited effectiveness in long-term trials. LAGB replaced the VBG and was responsible for a significant increase in the number of procedures performed after 2000. The first banding device, the LAP-BAND, was approved for use in the United States in 2001. A second device, the REALIZE band, was approved in the United States in 2007. In contrast to previous devices, the diameter of these bands is adjustable by way of their connection to a reservoir that is implanted under the skin. Injection or removal of saline into the reservoir tightens or loosens the band's internal diameter, respectively, thus changing the size of the gastric opening. Because there is no rerouting of the intestine with LAGB, the risk for developing nutritional deficiencies is entirely dependent on the patient's diet and eating habits. More recently, the laparoscopic sleeve gastrectomy (LSG) has replaced the LAGB as the most commonly performed operation among academic medical centers, accounting for 61% of all procedures in 2014 [15]. In this procedure, the stomach is restricted by stapling and dividing it vertically and removing approximately 80% of the greater curvature, leaving a slim "banana-shaped" remnant stomach along the lesser curvature. However, unlike the VBG or LAGB, removal of a portion of the stomach results in changes in hormonal metabolism that are similar to the restrictive–malabsorptive procedures described below.

B Restrictive–Malabsorptive Surgeries

The restrictive–malabsorptive bypass procedure combines the elements of gastric restriction and selective malabsorption. The RYGB is the most commonly performed procedure in this class. It involves formation of a 10- to 30-mL proximal gastric pouch by either surgically separating or stapling the stomach across the fundus. Outflow

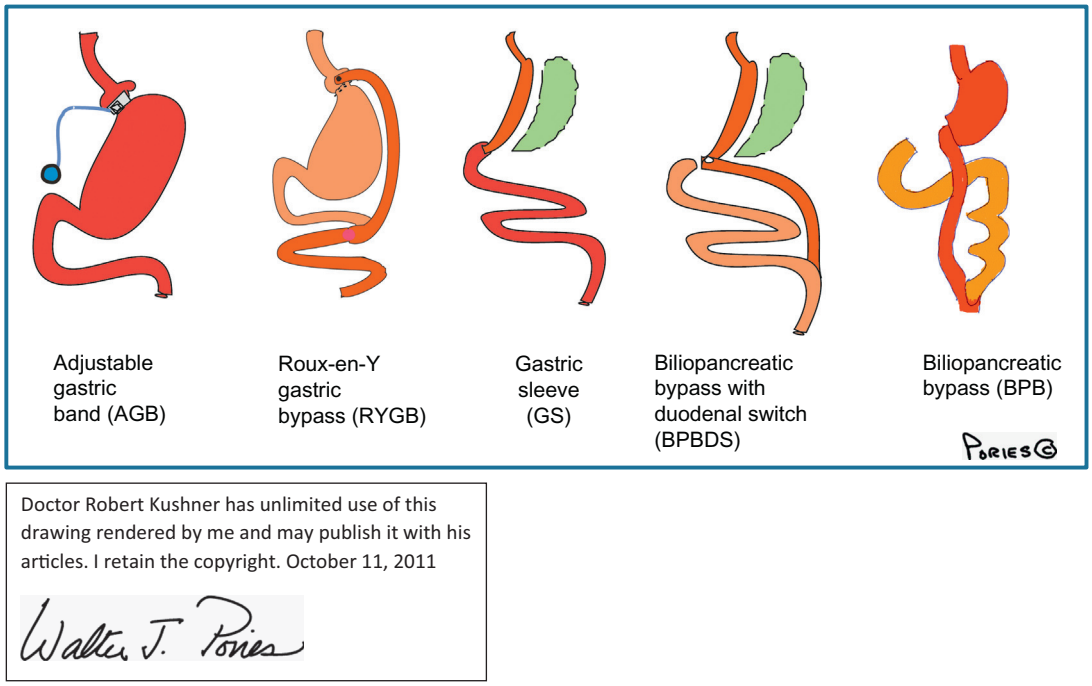

FIGURE 23.1 The five commonly accepted bariatric operations. *Reproduced with permission from Walter D. Pories.*

from the pouch is created by performing a narrow (10 mm) gastrojejunostomy. The distal end of jejunum is then anastomosed 50–150 cm below the gastrojejunostomy. "Roux-en-Y" refers to the Y-shaped section of small intestine created by the surgery; the Y is created at the point where the pancreobiliary conduit (afferent limb) and the Roux (efferent) limb are connected. "Bypass" refers to the exclusion or bypassing of the distal stomach, duodenum, and proximal jejunum. RYGB may be performed with an open incision or laparoscopically.

C Malabsorptive Surgeries

There are two malabsorptive procedures. In the BPD, a subtotal gastrectomy is performed, leaving a much larger gastric pouch compared with the RYGB. The small bowel is divided 250 cm proximal to the ileocecal valve and connected directly to the gastric pouch, producing a gastroileostomy. The remaining proximal limb (biliopancreatic conduit) is then anastomosed to the side of the distal ileum 50 cm proximal to the ileocecal valve. In this procedure, the distal stomach, duodenum, and entire jejunum are bypassed, leaving only a 50-cm distal ileum common channel for nutrients to mix with pancreatic and biliary secretions. The biliopancreatic diversion with duodenal switch (BPDDS) is a variation of the BPD that preserves the first portion of the duodenum. In this procedure, a vertical subtotal gastrectomy is performed and the duodenum is divided just beyond the pylorus. The distal small bowel is connected to the short stump of the duodenum, producing a 75- to 100-cm ileal–duodenal "common channel" for the absorption of nutrients. The other end of the duodenum is closed, and the remaining small bowel is connected onto the enteral limb approximately 75–100 cm from the ileocecal valve.

IV CLINICAL ASPECTS

A Weight Loss

Several meta-analyses and systematic reviews of bariatric surgery outcomes have been conducted [16–18]. In general, weight loss is greatest with the malabsorptive procedures (BPD and BPDDS), followed by the restrictive–malabsorptive procedure (RYGB), the LSG, and least with the restrictive LAGB procedure. As compared to standard care, differences in BMI levels from baseline at year 1 are $-11.3\ kg/m^2$ for BPD, $-9.0\ kg\ m^2$ for RYGB, $-10.1\ kg/m^2$ for LGS, and $-2.4\ kg\ m^2$ for LAGB [19]. Weight loss at 2–3 years following a surgical procedure varies from a mean of 20–34% of total weight depending on the procedure. The trajectory of weight loss also differs between procedure types. Whereas the rate of weight loss is slower with LAGB, with maximal weight loss achieved after 2 or 3 years, maximal weight loss with RYGB and LSG is achieved at 12–18 months [20,21].

B Effects on Comorbidities

A systematic review and meta-analysis of randomized controlled trials and nonrandomized controlled studies comparing bariatric surgery versus no surgery showed that surgery was associated with a reduced odds ratio (OR) risk of all-cause mortality (OR = 0.55), cancer (OR = 0.74), cardiovascular events (OR = 0.71), and stroke (OR = 0.66) [22]. Significant improvement in multiple obesity-related comorbid conditions has been reported, including type 2 diabetes, hypertension, dyslipidemia, obstructive sleep apnea, and quality of life [23]. The beneficial effect of weight-loss surgery on type 2 diabetes is particularly striking [24–26]. Several randomized controlled studies have demonstrated greater rates of partial or complete disease remission, and reduced use of antidiabetes medications after 3 years of follow-up [27,28]. Rates are greater following BPD, RYGB, or LSG than following LAGB, and the extent of remission is influenced by the amount of weight loss, weight regain, duration of diabetes, and the presurgery hypoglycemic therapy requirements [29]. The mechanisms of the greater antidiabetic effect following RYGB are primarily thought to involve caloric restriction and enhanced release of the incretins GLP-1 and glucose insulinotropic polypeptide, affecting both insulin secretion and sensitivity [30].

V PREOPERATIVE ASSESSMENT

A Overview

Guidelines for the perioperative nutritional, metabolic, and nonsurgical support of patients who undergo bariatric surgery have previously been published [8,31–33] along with "best practice" recommendations [34]. All patients who are considering weight-loss surgery should undergo a comprehensive assessment by a multidisciplinary team of health care providers that includes a physician, RD, and mental health care professional [8,31]. Preparation for surgery commonly spans 3–12 months depending on the patient's medical condition and criteria for insurance approval. During the preoperative process, patients are typically instructed on healthy eating and physical activity patterns, behavioral strategies to implement the lifestyle changes, and the importance of stress reduction and social support for long-term success. Specific dietary and nutritional recommendations pertinent to the surgical procedures include use of protein supplements, consumption of multiple meals and snacks, and slowing the rate of eating. Patients are seen either individually or in small groups. Many centers offer panel discussions between candidate patients and patients who have already undergone a procedure to provide a peer-to-peer discussion of risks, benefits, and challenges of life after bariatric surgery.

B Indications for Bariatric Surgery

According to the 1991 National Institutes of Health Consensus Development Conference Panel on bariatric surgery [3], patients with a BMI $\geq$40 kg/m^2 or those with a BMI $\geq$35 kg/m^2 who have associated high-risk comorbid conditions such as cardiopulmonary disease or type 2 diabetes could be considered surgical candidates. These indications have been reaffirmed by updated guidelines [8,31]. Consequently, Medicare and other third-party insurance payers have followed these criteria for reimbursement qualifications. The LAP-BAND system (Apollo Endosurgery, Inc, Austin, TX), one of the LAGB products used in the United States, has been approved by the US Food and Drug Administration for those with a BMI $\geq$30 kg/m^2 with a related health condition. In addition to BMI and the presence of comorbid conditions, other patient-related factors, such as psychosocial health, adherence, expectations, and past weight loss attempts, are taken into consideration. Contraindications include an extremely high operative risk, active substance abuse, or a major psychopathological condition such as major depressive disorder, schizophrenia, or bulimia.

C Medical Assessment

All patients need to undergo a comprehensive medical assessment prior to bariatric surgery. The purpose is to identify and optimally control medical problems prior to surgery. Patients with symptoms suggestive of obstructive sleep apnea (loud snoring, nighttime awakening, and morning and daytime sleepiness) should undergo a polysomnogram with initiation of continuous positive airway pressure (CPAP) if necessary. Patients with signs and symptoms of coronary artery disease or at high risk for cardiovascular disease should have a cardiac stress test performed. The two most common comorbidities that require preoperative control are diabetes and hypertension. Many patients may require initiation of medications to control blood glucose and blood pressure levels, respectively. Patients should discontinue cigarette smoking at least 2 months prior to surgery to reduce respiratory complications. Due to the rapid change in patients' medical conditions after surgery, patients need to be closely managed postoperatively for medication dose adjustments.

D Preoperative Nutrition Assessment

Dietary management of the bariatric patient begins preoperatively. All potential surgery candidates should meet with a RD to undergo a nutrition evaluation. The RD should determine if a patient meets the medically accepted criteria for bariatric surgery, educate the patient

on potential health and risk benefits of surgery, and determine the patient's ability to incorporate nutritional and behavioral changes. The nutrition evaluation is also used to determine a patient's education, existing medical conditions, and any potential physiological behaviors that could negatively impact the patient's weight loss after surgery. The RD should evaluate current diet, past weight loss attempts, nutritional deficiencies, or any disordered eating patterns including triggers to weight gain or challenges to following a healthy diet. Additionally, the dietitian should assess a patient's motivation for behavior change and social or economic concerns. Understanding the patient's expectations, knowledge, and self-efficacy regarding the postsurgery diet will also help the dietitian develop a nutrition prescription to set presurgery nutrition goals. The components of a nutritional assessment are outlined in Table 23.1.

Micronutrient deficiencies are common among patients with severe obesity [35,36]. Following a malabsorptive weight loss surgery, patients will be at higher risk for greater nutrient losses. Therefore, it is necessary to evaluate the patients' nutritional laboratory tests prior to surgery, specifically 25 (OH) vitamin D, calcium, thiamin, iron, and vitamin B_{12}. Deficiencies found in these nutrients should be corrected prior to surgery [8,31].

E Psychological Assessment

Psychological assessment should be performed by a licensed mental health professional prior to surgery. The evaluation serves two purposes: identification of potential contraindications to surgery, such as substance abuse or poorly treated depression or schizophrenia, and identification of potential postoperative problems, such as inability

TABLE 23.1 Presurgery Nutrition Evaluation

1. Reason for referral, anthropometric measures (weight/height/BMI)
2. Patient goals and expectation
   a. Patient's stated goals
   b. Reasons patient is seeking weight loss
   c. Patient's expectations regarding weight loss and surgery
3. Medications/abnormal nutrition labs
4. Weight history
   a. Onset of weight gain/onset of obesity
   b. Lowest/highest adult weight
   c. Family history of obesity
   d. Triggers for weight gain
      i. Life events, medications, postpartum weight retention, menopause, smoking cessation, work schedule, lack of nutrition knowledge, "emotional" eating behaviors
5. Prior weight loss efforts
   a. Commercial programs, medically supervised weight loss program, previous RD counseling, self-imposed diets, or previous history of bariatric surgery
6. Current diet intake
   a. Who shops/prepares food at home
   b. Diet recall: (24 h recall, food frequency)
   c. Food allergies/intolerances
   d. Supplement use
7. Patterns/habits
   a. Skips meals, unplanned snacks
   b. Fruit and vegetable intake
   c. Intake of highly processed foods or beverages
   d. Frequency of eating out/ordering in
8. Physical activity
   a. Time spent in sedentary actives (computer, TV, etc.)
   b. Work-related activity
   c. Planned exercise (type and frequency)
9. Assess
   a. Motivation for healthy eating
   b. Understanding of post-op diet, supplementation
   c. Ability and willingness to commit to all pre- and postsurgery appointments
   d. Need for additional nutritional education, support, pre-/post-op counseling
   e. Economic/financial ability for self-care following surgery (can the patient afford supplements, is access to healthy foods available?)

to adhere to behavioral changes needed for a successful outcome. Patients presenting with depressive symptoms, anxiety, low self-esteem, or relationship issues often benefit from preoperative intervention and/or a delay in surgery.

F The Role of Preoperative Weight Loss

In an effort to improve postoperative outcomes such as weight loss, diet adherence, and improved nutritional status, some bariatric surgery centers and insurance companies require patients to begin changing diet behaviors and striving for weight loss prior to surgery. The potential benefits of weight loss prior to surgery include acclimating to a restricted diet, increasing knowledge of healthy food choices, reduction in comorbidities, and a reduction in liver volume [37]. The purpose to reduce liver volume and intraabdominal fat is to help decrease surgery time and reduce comorbidities [38]. A 10% weight loss prior to surgery has been associated with decreased surgical complications and reduced hospital stays [39]. However, the research to support mandatory weight loss prior to surgery is limited and conflicting. The National Institute of Health does not require a mandatory weight loss for surgery.

VI POSTOPERATIVE MANAGEMENT

A Medical and Surgical Considerations

Patients who have undergone weight-loss surgery require ongoing postoperative care. Medical monitoring includes frequent assessment of preexisting comorbid conditions along with evaluation for postoperative complications. Patients with diabetes need to closely monitor their blood glucose levels because rapid resolution of diabetes frequently occurs following the RYGB, LSG, and BPDDS procedures. Antidiabetes medications are commonly reduced by half upon discharge from the hospital, and sulfonylurea agents—medications that may cause hypoglycemia—are discontinued entirely. Further reductions in antidiabetes medications are made based on glucose control. Similarly, antihypertensive medications may need adjustment as blood pressure falls. Diuretic agents are discontinued upon discharge from the hospital because they may lead to dehydration and electrolyte abnormalities. Patients with obstructive sleep apnea will frequently experience an improvement in sleep quality and reduction in the apnea hypopnea index. A repeat sleep study and retitration of CPAP is often performed 6–12 months postoperatively. Other preexisting medical problems, such as osteoarthritis, gastroesophageal reflux disease, urinary incontinence, asthma, heart disease, and hyperlipidemia, are managed according to symptoms or laboratory values [8,31].

Postoperative surgical care is necessary to ensure wound healing, surveillance for surgical complications, and, for patients who underwent LAGB, periodic tightening of the adjustable band. Food entrapment or vomiting suggests that the band is too tight and a need for loosening. Complications of the LAGB may include band slippage or erosion or fracture of the port and tubing. Complications from the RYGB procedure may include stenosis of the gastric outlet secondary to ulceration or scaring of the gastrojejunal anastomosis. These complications are routinely treated by prescription of an acid blocker and endoscopic dilation under light sedation. Intestinal obstruction can occur after the RYGB or malabsorptive operations and requires urgent surgical intervention. For these reasons, all patients should follow up with a physician who is knowledgeable about bariatric surgery.

B Nutrition Care

Follow-up of the nutritional status of the postbariatric surgery patient is essential for successful outcomes. Nutrition management after surgery has been shown to be a significant factor in postoperative weight loss. It is recommended that a follow-up period of up to 5 years is established to help prevent the development of nutrient deficiencies as well as weight regain [8]. Regular intervals of patient-provider contact can help patients achieve significant weight reduction and optimal health status. Conversely, failure to make follow-up appointments is associated with poorer weight loss and postoperative complications [32,40,41].

To ensure continuity of care for patients, it is important that dietitians provide ongoing MNT postoperatively. The primary focus of nutritional counseling following surgery is to facilitate weight loss while reducing nutritional deficiencies.

C Diet Progression Postsurgery

The postsurgery diet is a staged progression approach based on nutritional needs of the patient that will change according to the weight loss phase. This multiphase diet progression helps the patient transition from liquid to solid foods as nutritional needs and tolerances evolve. As there are a wide variety of diet protocols, there is no standardization of diet stages or diet progression. However, the general guidelines for diet stages post-RYGB and post-VSG are the same. Dietary guidelines for post-LAGB follows the same format, but progression through the diet stages and time spent on each diet phase may vary depending on surgery center. Therefore, it is crucial that all patients are followed frequently in the early

postsurgery months. Dietary progression is typically advised at the discretion of the dietitian [40].

Immediately following bariatric surgery, food intake is typically held until the GI tract function returns. When feeding is resumed, patients generally begin on a liquid diet before slowly progressing to solid foods as tolerated. Tolerance of food volume and consistency varies among patients who have undergone bariatric surgery, even among patients who have had the same procedure. It is important for all patients to be monitored frequently in the early stages after surgery.

1 Diet Stage 1: Liquid Diet

Clear Liquid Diet. The initial diet stage following the first 24–48 hours after bariatric surgery is a clear liquid diet. During this stage, GI motility is restored so these liquids must leave minimal residue in the GI tract. Patients are initially started on a clear liquid diet consisting of noncarbonated, sugar-free, or low sugar products such as water, coffee, tea, broth, sugar-free gelatin or ice pops, or any beverage sweetened with a sugar substitute [8,41].

Full Liquid Diet. Patients are progressed into a full liquid diet as tolerated. All liquids should remain noncarbonated, sugar-free, or low sugar. Patients may begin to incorporate beverages such as nonfat or low-fat milk, milk alternatives such as soy or almond milk, thin/strained soups with no solids such as tomato soup, protein drinks made with whey, whey isolate or soy protein powder, and yogurt. The progression into full liquids is intended to increase food tolerance as well as introduce texture and residue into the GI tract while providing patients with more energy and nutrients. Patients are encouraged to consume a minimum of 48–64 oz of fluids per day consisting of both clear and full liquids. Patients are advised to follow this diet stage for at least 10–14 days out of surgery [8,41].

2 Diet Stage 2: Pureed Diet

Approximately 2 weeks after surgery, patients will progress into a pureed food stage. Foods in this stage increase the gut tolerance of gastric residue from solute and fibers. Protein is emphasized and patients are encouraged to consume protein at three to six small meals over the course of the day. Protein supplements in the form of whey, whey isolate, or soy protein drinks are encouraged to further increase protein consumption. Tolerance for protein foods may be limited to only a few tablespoons at each meal or snack. Foods in this stage should be blended with water (or other liquid) to result in a pureed consistency resembling that of applesauce or baby food.

3 Diet Stage 3: Soft Diet

As pureed food is tolerated, patients are encouraged to incorporate soft foods. These soft foods are those that are easy to chew and will easily pass through the modified gastric pouch into the jejunum (RYGB) or flow easily through the modified stomach (VSG). Patients will continue to consume 48–64 oz of clear liquids per day, but will begin adding in soft, moist, diced, ground or pureed protein sources as tolerated. These foods include protein sources such as egg or egg whites, ground meats/ground poultry, soft fish, cooked beans, cottage cheese, low-fat cheese, and yogurts. This pureed and soft diet stage lasts approximately 10–14 days [33,40,42,43].

4 Diet Stage 4: Full or General Diet

After 2 weeks on the pureed/soft diet, patients are transitioned into a full or general diet as tolerated. If protein foods have been tolerated in the previous stages, patients are encouraged to add well-cooked or soft vegetables, soft and peeled fruits, and soft grains such as oatmeal or cooked cereals. In this stage, emphasis is placed on protein intake and restriction of certain dietary components such as concentrated sugar, fat, and refined carbohydrates such as pasta, rice, and bread. Throughout all diet phases, patients are encouraged to avoid eating and drinking simultaneously and to wait at least 30 minutes after meals to resume liquid consumption. A healthy diet consisting of lean proteins, fruits and vegetables, and whole grains should be consumed. Adequate hydration and fiber from sources such as fruits, vegetables, and whole grains will help prevent constipation [8,33,42,43].

D Nutrient Concerns and Supplementation

Nutritional deficiencies can and will occur in the bariatric patient for many reasons including inadequate food intake or poor food choices, malabsorption of nutrients from altered anatomy or persistent nausea, and vomiting that is common after all bariatric procedures in the early postoperative period. Nutrient deficiency will increase depending on the severity of the malabsorption. Due to the restrictive nature of LAGB and VSG surgeries, nutrition deficiencies are more like to occur due to inadequate or poor food choices. Because RYGB, BPD, and DS are malabsorptive, in addition to restrictive, micronutrient and macronutrient deficiencies are to be expected. Additionally, in these procedures, micronutrient deficiencies increase over time while the number of patients that are monitored decreases. Patients should also be made aware of additional deficiencies and concerns that may accompany poor attention to diet. Patients require lifelong vitamin and mineral supplementation regimes following

TABLE 23.2 Standard Postsurgery Supplementation and Dosage

Supplement	Recommended Dosage	
Multivitamin	1–2 daily	Should contain RDIs for all nutrients
		After 3 months, may switch to 1 daily
Calcium citrate	1200–1500 mg/day	Divided dose, do not exceed 600 mg in one dose
Vitamin D	3000 IU/day	Titrate to >30 ng/mL
Folic acid	400 mcg/day	Included in multivitamin
Elemental iron	18–27 mg/day	Not to be taken with calcium
		Taken with Vitamin C can increase absorption
Vitamin B_{12}	300–500 mcg/day	Oral, sublingual, or nasal
	1000 mcg/month	Intramuscular

Patients with deficiencies should be treated beyond these recommendations.
Source: From J.I. Mechanick et al., American Association of Clinical Endocrinologists, The Obesity Society, and American Society for Metabolic & Bariatric Surgery Medical guidelines for clinical practice for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient, 14 (Suppl. 1) (2008) 1–83; L. Allis et al., ASMBS Allied Health Nutritional guidelines for the surgical weight loss patient, SOARD 4 (2008) S73–S108.

these surgeries. It is essential that the multidisciplinary team provides guidance regarding nutritional supplementation to help patients maintain optimal health. A list of postoperative supplements and dosage can be seen in Table 23.2.

E Hydration

In the initial postoperative stages, some patients may find it difficult to adhere to the recommendation of 48–64 oz *of fluid per day. Due to decreased thirst, decreased appetite, and small pouch size, it may be difficult for patients to take in the necessary fluids, greatly increasing the risk of dehydration.* Patients should be aware of dehydration symptoms, including dark urine, headache, hard stools or constipation, dizziness, and extreme fatigue [8].

F Macronutrient Deficiencies

Macronutrients (protein, fat, and carbohydrates) supply energy and many essential nutrients and are needed in larger amounts in the diet to maintain homeostasis in the body. Nutritional deficiencies of macronutrients occurring in restrictive procedures such as VSG and LAGB are mostly a result of poor dietary intake as opposed to malabsorptive procedures such as BPD and DS, where as much as 25% of protein may not be absorbed [42,43].

Protein energy malnutrition (PEM) is the most severe macronutrient complication following malabsorptive procedures; protein malabsorption occurs in 7–21% of patients following BPD where up to 25% of protein is malabsorbed [43]. Reduced pouch capacity does not impact protein digestion as protein is digested in the small intestine; however, reduced capacity will impact overall protein intake. All bariatric patients are subject to increased PEM as a result of decreased dietary intake, anorexia, vomiting, food intolerance, fear of weight regain or decreased weight loss, and socioeconomic status [44].

PEM is typically detected 3–6 months following surgery and is mostly related to poor food intake and food intolerance to protein-rich foods. Patients should be regularly assessed for intake of protein-rich foods and protein supplements. Protein requirements for adults should be 10–35% of total energy. Patients who have undergone BPD or DS should consume 80–120 g protein per day. Patients who have undergone RYGB, VSG, or LAGB should be consuming at least 60 g or more per day [44].

Patients should be encouraged to consume protein-rich food sources as well as liquid protein supplements from whey, whey isolate, casein, milk, egg whites or soy protein sources. Patients should be cautioned that whey protein concentrates may contain significant sources of lactose that may not be tolerated among patients after RYGB. Lactose-free protein sources such as soy, egg whites, and whey isolates may be preferable to these patients [45]. Tolerance of protein foods tends to increase after the first year of surgery [42].

At this time, there are no specific recommendations for carbohydrate intake for the bariatric patient. Patients are encouraged to consume 50–130 g of carbohydrates daily to meet the requirements for brain activity and for glucose to fuel the central nervous system [42,46]. Patients should incorporate nutrient-dense, complex carbohydrates and fiber as whole grains, legumes and beans, low-fat dairy, and fruit. Patients should also be

encouraged to eliminate and avoid simple carbohydrates and concentrated sugars. Patients who have undergone RYGB may exhibit symptoms of dumping syndrome such as nausea, dizziness, perspiration, cramping, and diarrhea after consumption of refined carbohydrates [47]. Intake of some foods such as bread, rice, pasta, and soft-textured dough is typically difficult to tolerate following surgery [44].

As is the case with carbohydrates, there are no specific recommendations for fat intake after bariatric surgery. Overall, a low-fat diet is advised, and patients should be encouraged to consume unsaturated fats and essential fatty acids. Malabsorption of fat is greater in patients who have undergone BPD and DS, as these procedures can result in as much as 72% of fat being malabsorbed. Patients who undergo these malabsorptive procedures are at greater risk for deficiencies in fat-soluble vitamins such as vitamins A, D, E, and K.

G Micronutrient Deficiencies

Nutrient deficiencies can occur following malabsorptive and restrictive procedures. Following the RYGB, BPD, and DS procedures, key micronutrients are lost after bypassing the stomach, duodenum, and portions of the jejunum and ileum. Patients are at risk of depletion of thiamin, iron, folate, vitamin B_{12}, calcium, and vitamin D [48]. Along with supplementation of these nutrients, patients should also incorporate a daily multivitamin. Not all multivitamins are formulated the same, however. Recommendations postsurgery should include a daily multivitamin that meets the Daily Reference Intake (DRI) for adults. All postsurgery multivitamins should include 100% of the nutrients as listed in Table 23.3.

TABLE 23.3 DRI for Postoperative Multivitamins

	Adult Males	Adult Females
DRI		
Vitamin K (μg)	120	90
Biotin (μg)	30	30
Zinc (mg)	11	8
Thiamin (mg)	1.2	1.1
B_{12} (μg)	2.4	2.4
Folic acid (μg)	400	400
Iron (mg)	8	18
Copper (mg)	0.7	0.7

All multivitamins are not formulated similarly. All post-op multivitamins should contain 100% of these nutrients.
Source: From Dietary Reference Intakes (DRIs), www.nap.edu

Nutrient deficiencies may also emerge in LAGB or VSG procedures, though there are no changes to the absorptive pathway. Low nutrient intake, poor food choices, food intolerances, and limited portion sizes may contribute to micronutrient deficiencies in patients who undergo any bariatric surgery procedure. The absorption sites for micronutrients are shown in Fig. 23.2.

H Thiamin

Thiamin (vitamin B_1) is a water-soluble vitamin that is absorbed in the proximal small intestine. Thiamin deficiencies have been seen in up to 49% of patients following bariatric surgery [43]. Thiamin is not stored in large amounts in the body and levels can drop quickly from excessive vomiting, poor food choices, or limited food intake [48]. Deficiency in this vitamin mainly affects the central nervous system and can result in cardiac and neurologic complications resulting in the development of Wernicke's encephalopathy (leading to Wernicke–Korsakoff syndrome) and beriberi. Wernicke's encephalopathy is a neurologic complication characterized by ataxia, nystagmus, double vision, disorientation, and confusion. It can be seen in patients as early as 1 month postoperatively and is most commonly precipitated by intractable vomiting. Wet beriberi can affect the cardiovascular system and eventually lead to heart failure. Symptoms include tachycardia, shortness of breath, and swelling of the lower legs. Dry beriberi affects the nervous system and can lead to loss of muscle function, paralysis, and mental confusion.

All patients who have undergone bariatric surgery, especially those who have had malabsorptive procedures, should be monitored and supplemented with a B-vitamin complex or multivitamin as early as 2 weeks postoperatively to prevent thiamin deficiency [48,49]. Recommendations for maintenance of thiamin levels is 3 mg daily, which can be included in a multivitamin; patients who are experiencing extreme nausea and vomiting may require additional thiamin supplementation.

I Iron

Iron deficiency is more commonly found after RYGB and BPD/DS procedures due to the altered anatomy of the intestinal tract. Iron is absorbed throughout the small intestine, but is most efficiently absorbed in the duodenum and proximal jejunum, which is bypassed following these procedures. Deficiency can also result from decreased food intake and/or lack of gastric acid. The hypoacidic environment limits the conversion of iron into its absorbable ferrous form [42].

Malabsorption of iron coincides with a decreased intake of iron-containing foods, such as red meats, grains,

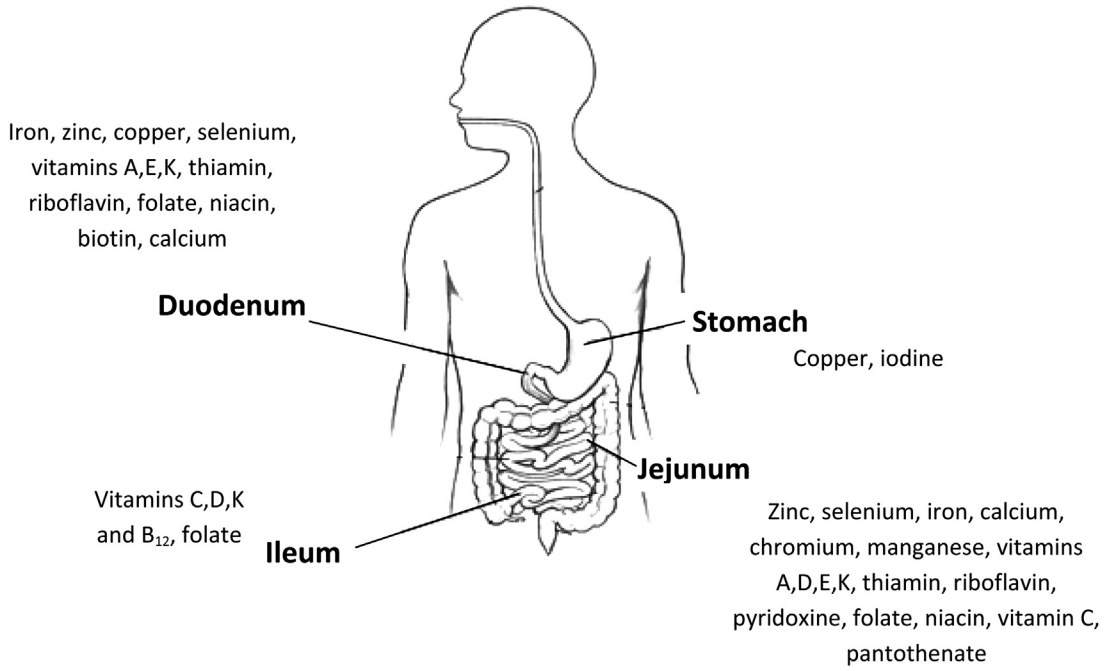

FIGURE 23.2 Micronutrient absorption sites within the GI tract.

and vegetables, which may be difficult for patients to tolerate and are typically avoided during the first few months following surgery. Iron deficiency is seen in 20–55% of RYBG patients, 0–18% of VSG patients, 13–62% of BPD patients, 8–50% of DS patients, and 14% of LAGB patients [50].

Iron deficiency is the main cause of anemia for bariatric surgery patients but anemia may also result from low levels of vitamin B_{12} and folate. Symptoms may include fatigue and reduced capacity for exercise tolerance. To prevent iron deficiency, all patients should be evaluated prior to surgery to determine any preexisting diminished iron stores as obesity-related inflammation interferes with iron availability [43,51].

Postsurgical patients should supplement with daily multivitamin–mineral supplements. It is recommended that 40–65 mg/day of elemental iron (alone or included in a multivitamin) is needed to maintain normal iron levels [8]. Menstruating women and those individuals who are at a higher risk of developing anemia should take an additional 50–100 mg/day [8,42]. Patients may find it difficult to adhere to oral supplementation due to GI side effects (constipation and cramping) or nutrient interactions. Iron supplementation can be inhibited in the presence of calcium supplementation, especially calcium carbonate, calcium citrate, or calcium phosphate. Vitamin C may increase the absorption of iron; patients should increase their intake of citrus fruits or vitamin C supplements. The risk for iron deficiency decreases over time as the patient is able to increase food intake and experience fewer food intolerances [8,42,43].

J Vitamin B_{12}

Malabsorptive and restrictive bariatric surgical procedures interfere with several key processes of vitamin B_{12} absorption. Due to changes in hydrochloric acid (HCl) production and reduced availability of intrinsic factor (IF), B_{12} absorption is impaired [42,43]. Post-RYGB, patients no longer have the ability to release vitamin B_{12} from protein foods because pepsinogen cannot be converted into pepsin due to the lack of HCl. Decreased production of IF by the parietal cells in the stomach will also prevent vitamin B_{12} from being absorbed. The parietal cells that excrete acid and IF, as well as the cells that excrete pepsinogen, are located primarily in the fundus and body of the stomach and is bypassed after RYGB.

Deficiency in vitamin B_{12} and folate is seen more prevalent with RYGB and BPD/DS patients than with VSG or LAGB patients. These procedures leave more of the stomach intact, producing more gastric acid and a subsequent increased availability of IF. However, these patients may still become deficient in B_{12} stores as a result of low tolerances of animal protein foods. Deficiency prevalence ranges from 7% to 70% by 3 years postoperatively [42,51]. Patients may begin to exhibit signs of B_{12} deficiency, including numbness or tingling in

the lower extremities, changes in gait or motor skills, loss of concentration, memory loss, or disorientation.

Typically, only those patients receiving RYGB and BPD/DS are supplemented with vitamin B_{12}, although all patients undergoing bariatric surgery should be routinely screened. Recommendation for maintenance levels of B_{12} is 1000 mcg/day. This can be given over a variety of courses (daily, weekly, or monthly) and across multiple administrative routes (oral, intramuscular, nasal, or sublingual) as recommended [8,42,43].

K Folate

Folate absorption occurs in the proximal small intestine. Folate deficiency is not as commonly observed as with B_{12} or iron deficiency, but patients who become anemic should be screened for a folate deficiency. Folate deficiency may occur as a result of inadequate dietary intake, nonadherence with multivitamin supplementation, or malabsorption. Folic acid stores can begin to diminish within a few months postoperatively and will present with symptoms such as macrocytic anemia and mental confusion (e.g., forgetfulness or paranoia). Prevalence of folate deficiency has been reported in 0−40% of patients following surgery, and has been reported in 22% of VSG patients and 12% of RYGB following surgery [51−53].

Multivitamin and dietary sources such as dark green, leafy vegetables, fruits, organ meats, and fortified grain products can replenish folic acid stores in the body. Supplementations of folic acid are recommended at 200% of the daily value (DV), between 800 and 1000 μg/day, which is commonly found in most multivitamins. This dose can correct or prevent folate deficiency in most patients [52]. Supplementation exceeding >1000 μg/day is not recommended as this can potentially mask a B_{12} deficiency. Folic acid deficiency in pregnant women is associated with an increased risk of neural tube defects in newborns. Women of childbearing age or who are seeking to become pregnant postbariatric surgery should monitor their intake of folic acid [54].

L Calcium and Vitamin D

Calcium and Vitamin D are typically grouped together, as a deficiency in one or both of these may lead to metabolic bone disease, increased bone turnover, secondary hyperparathyroidism, undermineralized bone, and bone loss [55]. A reduction in bone mineral density and bone mineral content generally accompanies weight loss; it is crucial to differentiate between the effects of weight loss compared to the malabsorptive consequences of bariatric surgery. A deficiency of calcium and vitamin D may result from a decrease in calcium and vitamin D rich dairy foods related to intolerance, or by a reduction in intestinal absorption resulting from surgical bypass of the duodenum, proximal jejunum, and ileum. Calcium is absorbed in the duodenum and proximal jejunum and is dependent on vitamin D levels. Vitamin D is absorbed in the small intestine, which is altered following RYGB and BPD/DS. Although calcium and vitamin D deficiencies are more prevalent in patients undergoing malabsorptive procedures, patients who had a VSG and LAGB can still experience calcium and vitamin D deficiencies due to poor food intake, usually related to intolerances to dairy products [42,43].

While calcium and vitamin D deficiency following malabsorptive procedures is predictable, patients often present with unrecognized deficiencies preoperatively. Patients with a high BMI may be subject to develop vitamin D deficiencies related to decreased bioavailability of vitamin D due to enhanced uptake and clearance by adipose tissues, or underexposure to the sun. Patients with severe obesity should be screened for vitamin D deficiency and abnormalities in bone density prior to bariatric surgery [42,43,56].

Oral supplementation should be given to support optimal bone health and to correct for deficiency. Recommended supplementation of calcium is 1200−1500 mg/day coupled with a calcium-rich diet [8]. Calcium intake poses many challenges for patients. A limit of 600 mg of calcium should not be exceeded in a single dose. Calcium supplements should be taken separately, ideally 2 hours apart, from iron or iron-containing multivitamins as calcium can interfere with iron absorption. Calcium citrate has been shown to be superior to calcium carbonate as it is absorbed better in the low acidic environment postoperatively [43,57]. With reduced calcium levels, patients are at greater risk of developing osteoporosis and fractures due to rapid weight loss and decreased absorption. Periodic measurements of 25 (OH) vitamin D, parathyroid hormone, and calcium should be obtained to screen for deficiency along with monitoring of bone density [43,47].

Patients with severe vitamin D deficiency should be supplemented at 50,000 IU of ergocalciferol orally once to twice per week for 8−12 weeks. Patients who underwent a BPD/DS procedure may require daily supplementation [8,42].

M Vitamins A, E, and K

Surgical procedures that alter fat digestion will result in differing absorption and transport of fat-soluble vitamins [42]. Following BPD/DS, intestinal dietary fat absorption is decreased as a result of the limited absorption areas, delays in the mixing of gastric and pancreatic enzymes with bile in the ileum. As fat-soluble vitamins are absorbed in the jejunum and ileum, this increases the risk of deficiencies for postsurgery patients. Additional

supplementation of vitamin A may be required after the BPD/DS procedures. Guidelines for vitamin A supplementation are 10,000 IU per day for BPD/DS patients followed by screening at 6 and 12 months postoperatively [8]. If deficiencies are discovered, plasma retinol should be assessed [42].

Vitamin K and vitamin E deficiencies are rare following bariatric surgery procedures, especially among patients who are taking a daily supplement regimen of 1−2 multivitamins that contain vitamin K and E [43]. Deficiencies of vitamin K are more prevalent in BPD/DS patients; these patients require an additional supplementation of 300 mcg vitamin K daily [42,43]. Levels should be assessed if the patient is experiencing hepatopathy, coagulopathy, or osteoporosis [8].

N Zinc, Copper, and Trace Elements

Zinc, copper, and iron compete for the same transport molecules in the body. Therefore, there can be an imbalance of these minerals following surgery. These trace minerals are absorbed in the jejunum, and absorption is compromised following RYGB and BPD/DS. Symptoms of zinc deficiency include alopecia, delayed wound healing, skin lesions, immune deficiencies, and hypogeusia. Impaired zinc absorption is also associated with intestinal diseases such as Crohn's disease or pancreatic insufficiency. Zinc is dependent upon fat absorption for metabolism and may be compromised after surgery due to poor dietary intake and/or poor fat absorption, especially as zinc is found in animal products including red meat and poultry as well as dairy products. A protein-rich diet promotes zinc absorption by forming zinc-amino acid chelates (the more absorbable form of zinc). There is insufficient research to support specific recommendations regarding zinc supplementation, but it is recommended for patients following RYGB and BPD/DS. A daily multivitamin supplement containing the DV of zinc (8−11 mg) is adequate in preventing zinc deficiencies [8,43].

Deficiencies in copper present similarly to deficiencies of B_{12} and can result in anemia and myelopathy. Copper is absorbed by the stomach and duodenum, and the risk of copper deficiencies is greater when this anatomy is bypassed. In addition to the malabsorption from these surgeries, diarrhea may also cause excess loss of copper. In patients who present with symptoms or neuropathy but have normal levels of B_{12}, copper levels should be examined. Currently there is no consensus for copper supplementation. Daily multivitamin supplements containing the DV (2 mg) should be adequate in preventing copper depletion in postsurgical bariatric patients [47].

There are insufficient data supporting the need to monitor trace elements (selenium, magnesium, chromium, sulfur, boron, iodine, and fluoride) after bariatric surgery. However, as selenium is absorbed in the jejunum, levels should be checked in patients that have undergone BPD/DS. Selenium deficiency has also been shown to occur in patients who have undergone RYGB and VSG as well. Patients presenting with unexplained anemia, fatigue, persistent diarrhea, cardiomyopathy, or metabolic bone disease should be examined for trace element deficiencies. Daily supplementation with a multivitamin that contains these trace elements should be sufficient to prevent any deficiencies of trace elements [8,43,47].

O Dumping Syndrome

Dumping syndrome occurs as a result of rapid gastric emptying and GI and systemic responses to a meal. More common with RYGB, dumping syndrome occurs after the ingestion of high-sugar or highly processed carbohydrates. Due to the lack of the pyloric sphincter, a hypertonic solution forms in the jejunum following the ingestion of sugar and or highly refined carbohydrates. This will lead to a sudden distention of the jejunum and result in symptoms of early dumping syndrome. These symptoms, including abdominal pain and cramping, nausea and vomiting, flushing, tachycardia, and syncope, can appear within 10−30 minutes after eating [58,59].

Late-stage dumping syndrome may occur anywhere from 1 to 3 hours after eating. Symptoms include perspiration, feelings of anxiousness or weakness, shakiness or hunger, and difficulty concentrating. These symptoms are a result of an excessive insulin release. This is a result of a rapid absorption of glucose which triggers an exaggerated insulin release and results in rebound or reactive hypoglycemia.

Dietary recommendations for patients experiencing dumping syndrome will vary depending upon each patient's diet and severity of symptoms. Recommendations to relieve symptoms of dumping syndrome include eating small meals spread throughout the day to improve absorption and to decrease dramatic fluid shifts. Meals should be high in protein, contain moderate levels of fat and should be devoid of refined or "simple" carbohydrates. Foods containing naturally occurring sugars, such as fruits and dairy, do not typically contribute to dumping syndrome. Proteins and fats are hydrolyzed more slowly into osmotically active substances whereas simple carbohydrates in the forms of lactose, sucrose, and dextrose are hydrolyzed rapidly. Complex carbohydrates and foods high in fiber content slow GI transit time and increase viscosity, but caution should be taken to avoid ingesting large amounts of high-fiber foods until tolerated. If fiber supplements are instituted, patients should be cautious with large supplements in the form of pills, as gastric narrowing, especially

following the LAGB procedure, may make it difficult for supplements to pass through narrowed gastric openings or if the patient is experiencing dysmotility [58,59].

VII LONG-TERM CONCERNS

A Weight Regain

Although clinicians commonly see bariatric surgery patients regain some weight postoperatively, the prevalence and incidence of weight regain has not been well characterized. The underlying factors that influence weight regain following bariatric surgery are multifactorial and include endocrine/metabolic alterations, anatomic surgical failure, nutritional indiscretion, mental health issues, and physical inactivity. The extent and significance of these factors is currently uncertain and likely varies between individuals and the operative procedure performed. Using cross-sectional data, weight regain has been estimated to occur in 20–35% of patients, depending on the procedure performed and duration of time following surgery [33]. Table 23.4 provides a categorical list of potential etiologies that should be explored with all patients who present with weight regain. The physiological and behavioral (diet and physical activity) causes are common to surgical and nonsurgical patients. Depending on the patient's age and gender, a thorough history should be performed that reviews all of these reasons followed by appropriate counseling. This section will focus on the dietary and nutritional considerations.

Immediately following surgery there is a reduction in a caloric intake due to a smaller gastric capacity, diminished hunger, and increased satiety brought about by the anatomical and metabolic changes discussed above. Over time, however, caloric intake is less restrained, which contributes to postoperative weight regain. In the Swedish Obese Subjects study [60], mean daily intakes of 2900, 1500, 1700, 1800, 1900, and 2000 kcal/day, respectively, were reported at baseline and 6 months, 12 months, 2 years, 3 years, and 4–10 years after surgery. These increases in caloric intake likely contribute to weight regain, which often begins in the second postoperative year. Dietary nonadherence, or the consumption of high calorie foods and beverages, may also contribute to the higher caloric intake. The results from a postoperative behavioral survey among 203 patients revealed a positive correlation between the magnitude of weight regain and consumption of large quantities of food in the evening or night, eating large quantities of high fat foods, and eating out more frequently [61]. In a case series of 289 patients who underwent RYGB, 23% of the population demonstrated dietary nonadherence and a continuation of presurgical eating patterns, leading to suboptimal weight loss, weight regain, or both [62]. These studies substantiate the importance of diet quality and caloric intake as causative factors for postsurgical weight regain.

TABLE 23.4 Etiological Factors for Weight Regain Following Bariatric Surgery

Anatomical
LAGB malfunction or mismanagement
Band or port breakage, band too lose
RYGB
Pouch enlargement
Gastrojejunal anastomosis dilation
Gastrogastric fistula
Physiological
Pregnancy
Menopause
Weight-gaining medications
Smoking cessation
Endocrine disorder: hypothyroidism, Cushing's disease
Intestinal or hormonal adaptation
Behavioral
Dietary
Unhealthy eating patterns, grazing, nibbling, mindless eating
Consumption of high-energy foods and beverages
Loss of dumping syndrome symptoms
Loss of control over urges, binges
Reduced vigilance
Physical activity
Reduced leisure time activity
Increased sedentary behaviors
Insufficient moderate- and vigorous-intensity exercise
Development of physical limitations to exercise

Grazing, defined as repeated episodes of consumption of smaller quantities over a long period of time with accompanying feelings of loss of control, has been identified as a common high-risk eating pattern after bariatric surgery [63,64]. Studies have shown that both preoperative and postoperative grazing behaviors independently predict poorer postsurgical weight loss [64].

Maladaptive dietary patterns have also been linked to other lifestyle behaviors that may impact eating habits among surgical patients. In data from the National Weight Control Registry collected at entry and 1 year follow-up,

surgical participants reported lower dietary restraint than nonsurgical participants [65]. Odom et al. [66] surveyed 203 patients (24.8% response rate) from a single center after a mean follow-up of 28.1 ± 18.9 months after RYGB. Seventy-nine percent of patients reported weight regain. Independent predictors of significant weight regain were lack of control of food urges (OR = 5.1), concerns over alcohol or drug use (OR = 12.74), lowest self-reported well-being scores (OR = 21.5), and no follow-up visits (OR = 2.60). In summary, attention to dietary nonadherence and maladaptive eating patterns is an important target to prevent postsurgery weight regain. Additional studies are needed to better understand the frequency, etiology, and treatment for weight regain.

VIII CONCLUSION

All bariatric surgeries have nutritional risks and consequences. The surgical multidisciplinary team is necessary to assess and prepare patients medically, nutritionally, and psychologically in an effort to reduce risks and improve outcomes following surgery. Bariatric surgery necessitates dietary modification of food texture, consistency, volume (both of solids and liquids), frequency and duration of meals, and adjustments for food intolerances, and potential nutrient deficiencies. Pre- and postoperative nutrition evaluations made by a RD will guide treatment recommendations and provide valuable information regarding both short- and long-term nutritional and behavioral treatment goals. All team members should be aware of postsurgery nutritional implications and environmental/behavioral factors that influence postsurgical weight loss, overall health and long-term weight maintenance.

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Chapter 24

Behavioral Risk Factors for Overweight and Obesity: Diet and Physical Activity

Elisabeth M. Seburg[1], Melissa M. Crane[1,2] and Nancy E. Sherwood[1,2]
[1]HealthPartners Institute, Minneapolis, MN, United States, [2]University of Minnesota, Minneapolis, MN, United States

I INTRODUCTION

Understanding the determinants of obesity and developing appropriate prevention and treatment strategies require an in-depth examination of behavioral risk factors for obesity. The goal of this chapter is to review available data regarding the prevalence of overweight and obesity and the modifiable dietary and physical activity behavioral determinants of obesity that are potential targets for obesity prevention and treatment interventions. We begin with an examination of dietary factors that contribute to the development of overweight and obesity. Multiple factors that influence food intake will be discussed, including total energy intake, specific eating patterns, and environmental and societal influences. We then discuss physical activity factors, including exercise self-efficacy, social support, and access to exercise opportunities. The review will focus on behavioral risk factors for obesity in both children and adults.

II OBESITY AND OVERWEIGHT

Obesity is a significant problem that affects children, adolescents, and adults across gender, race, and socioeconomic strata [1–8]. As shown in Fig. 24.1, the prevalence of overweight and obesity provided by the objectively assessed weights during the National Health and Nutrition Examination Surveys (NHANES) show that the percentage of obese adults has increased over the last five decades [9]. Results from NHANES 2011–12 indicate that obesity rates may have stabilized with 68.5% of U.S. adults aged 20 years and over classified as either overweight or obese, with an estimated 34.9% considered obese (body mass index, BMI $\geq$30 kg/m^2), and 6.4% in the obese class 3 (BMI $\geq$40 kg/m^2) category [8]. Although there has been a stabilization of the overall prevalence of obesity, some groups including women over the age of 60 have seen continued increase in prevalence in recent years [8].

Prevalence of obesity among children and adolescents has also increased dramatically since the mid-1960s (Fig. 24.2) [9]. Results from NHANES 2011–12 indicate that 31.8% of U.S. children and adolescents aged 2 through 19 years are overweight ($\geq$85th percentile of BMI for age and gender), with 16.9% in the obese ($\geq$95th percentile of BMI for age and gender) category. Similar to trends in adult obesity prevalence, data from the past decade suggest that overall obesity prevalence among children and adolescents has remained high, but relatively stable [8,10]. NHANES data from 2003–04 through 2011–12 reveal small, but nonsignificant, changes in obesity rates among children aged 6–11 years (18.8–17.7%) and adolescents aged 12–17 years (17.4–20.5%) over this time period. A notable exception is among preschool aged children (aged 2–5 years), among whom obesity rates decreased from 13.9% in 2003–04 to 8.4% in 2011–12 [8]. A similar trend is observed in low-income preschool aged children (aged 2–4 years) monitored by the Pediatric Nutrition Surveillance System [11], with small declines in prevalence of obesity (15.2% in 2003 to 14.9% in 2010) and extreme obesity (2.2% in 2003 to 2.07% in 2010) [12]. The persistently high prevalence of obesity and overweight among children and adolescents remains of particular concern, given that childhood-onset overweight and obesity often tracks with adult obesity [13–15].

The prevalence of overweight and obesity has not increased consistently across racial, ethnic, and

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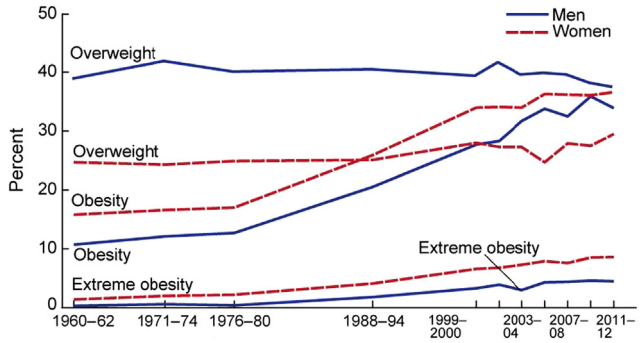

FIGURE 24.1 Trends in adult overweight, obesity, and extreme obesity among men and women aged 20–74: United States, selected years 1960–62 through 2011–12. *Notes*: Age-adjusted by the direct method to the year 2000 U.S. Census Bureau estimates using age groups 20–39, 40–59, and 60–74. Pregnant females were excluded. Overweight is BMI of 25 or greater but less than 30; obesity is BMI greater than or equal to 30; and extreme obesity is BMI greater than or equal to 40. *CDC/NCHS, National Health Examination Survey 1960–62; and NHANES 1971–74; 1976–80; 1988–94; 1999–2000; 2001–02, 2003–04, 2005–06, 2007–08, 2009–10, and 2011–12.*

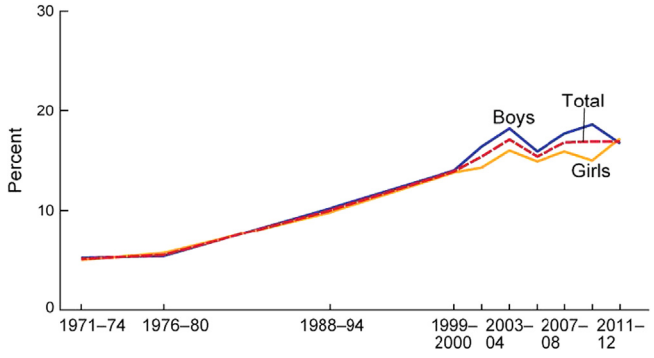

FIGURE 24.2 Trends in obesity among children and adolescents aged 2–19 years, by sex: United States, selected years 1971–74 through 2011–12. *Note*: Obesity is BMI greater than or equal to the sex- and age-specific 95th percentile from the 2000 CDC growth charts. *Fryar CD, Carroll MD, Ogden CL. Prevalence of Overweight and Obesity Among Children and Adolescents: United States, 1963–1965 Through 2011–2012. National Center for Health Statistics. September 2014. Available from: https://www.cdc.gov/nchs/data/hestat/obesity_child_11_12/obesity_child_11_12.pdf*

socioeconomic groups. Among both children and adults, Hispanic, non-Hispanic African American, and American Indian/Native Alaskan are at particularly high risk for obesity [10,16–20]. There is also evidence for an inverse relationship between socioeconomic status (SES) and obesity among women and children, although the relationship is not apparent among adult men [4,21,22]. In their recent systematic review of SES and overweight and obesity prevalence in children and adolescents in developed countries, Chung and colleagues conclude that there are significant differences in prevalence of overweight and obesity by SES group, with higher prevalence of overweight and obesity in low-SES children and adolescents in many countries [22]. In fact, several studies suggest the gap in obesity and overweight prevalence between U.S. children from low- and high-SES groups is widening [23–25]. Similarly, racial and ethnic disparities in obesity prevalence persist and may be increasing for some groups [26]. For example, among low-income preschool aged children, declines in obesity prevalence are seen for non-Hispanic White, non-Hispanic African American, Hispanic, and Asian/Pacific Islander children, but not for American Indian/Alaskan Native children [12,27].

The high prevalence of obesity and overweight among U.S. children, adolescents, and adults raises concerns about associated health risks across the lifespan [13], as well as intergenerational transmission of overweight and obesity risk [28]. There is substantial evidence of the persistence of overweight and obesity; childhood-onset of overweight and obesity tracks into adulthood, with youth who are overweight and obese more likely to be overweight and obese in adulthood [13,29]. Furthermore, parental obesity is a risk factor for obesity in childhood [30–33]. Sustained high levels of obesity and overweight in the U.S. population could lead to substantial increases in the number of people affected by obesity-related health conditions and premature mortality [34,35]. The health risks associated with obesity are numerous and include hypertension, type 2 diabetes mellitus, dyslipidemia, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and certain cancers (e.g., endometrial, breast, prostate, and colon) [36–44]. In children and adolescents, obesity is associated with health risks, such as cardiovascular risks (e.g., dyslipidemia and endothelial dysfunction [45–50]), slipped capital femoral epiphysis [51], obstructive sleep apnea [52], and decreased quality of life [53]. Children and adults who are obese are also adversely impacted by social bias and discrimination [54,55].

In addition to the many health consequences of excess body weight, there is a sizeable obesity-related economic burden attributable to individual health, costs to society due to lost productivity, and premature mortality and treatment costs [56–61]. According to a recent systematic review and meta-analysis, the per-person direct medical cost of obesity is an estimated $1901 in 2014 U.S. dollars, with the aggregate national cost of obesity estimated at $149.4 billion [61]. Obesity-associated health care costs are not limited to adults [62–66]. The lifetime incremental direct medical costs for an obese 10-year old, compared to a normal weight 10-year old who remains normal weight through adulthood, are an estimated $12,660–$19,630 in 2012 U.S. dollars [66]. This translates to an additional $9.4–14 billion in lifetime direct medical costs for this age cohort. Other costs of obesity include an estimated loss of $8.65 billion annually due to obesity-related absenteeism [67].

Obesity and overweight are multidetermined chronic problems resulting from complex interactions between genes and an environment characterized by energy imbalance due to sedentary lifestyles and ready access to an abundance of food [68]. Genetic research holds considerable promise for understanding the development of obesity and identifying those at risk for obesity, including via low resting metabolic rate, low level of lipid oxidation rate, low fat-free mass, and poor appetite control [69–71]. Recent epidemiological trends in obesity have also been linked to behavioral and environmental changes that have occurred in recent years. The higher proportion of fat and the higher energy density of the diet in combination with reductions in physical activity levels and increases in sedentary behavior have been implicated as significant contributors to the obesity epidemic [68].

III DIETARY INTAKE FACTORS

A Energy Intake

Laboratory experiments in animals and human clinical studies have repeatedly shown that the level of fat and energy intake in the diet is strongly and positively related to excess body weight. Examination of secular trends in self-reported overall energy intake, eating frequency, and energy density of diets suggest that trends of food consumption roughly parallel the pattern of obesity observed in the United States over the past 40 years [72–74]. Examination of trends in energy intake from nine consecutive NHANES show that energy intake increased beginning in 1976–80, and peaked in 2003–04 for most groups (1946 kcal/day to 2269 kcal/day). Among men, average daily energy intake increased from 2401 kcal/day in 1976–80 to a peak of 2701 kcal/day in 2005–06 ($p < 0.01$). Among women, average daily energy intake peaked in 2003–04, increasing from 1521 kcal/day in 1976–80 to 1885 kcal/day in 2003–04 ($p < 0.01$) [72]. Declines in energy intake were observed between the 2003–04 and 2009–10 surveys, with average daily energy intake decreasing from 2269 kcal/day to 2195 kcal/day [72]. The decrease in energy intake seen in U.S. adults roughly aligns with stabilizing obesity prevalence among adults between 2003 and 2012 [8]; it is possible that these apparent declines in energy intake contribute to the recent leveling off of obesity rates.

Secular trends in energy intake of children and youth have also been examined [75–80]. Data from NHANES found that mean energy intake remained relatively stable from the 1971–74 to 1999–2000 surveys, except for an increase among adolescent girls of 80 kcal/day [76]. Similarly, examination of data from NHANES 1988–94 through 2003–08 showed no increase in total energy intake over time among children aged 2–19 years [78]. In contrast to analyses that use NHANES baseline data, comparisons of the 1977–78 Nationwide Food Consumption Survey and 1989–91 Continuing Survey of Food Intakes by Individuals with the 2003–06 and the 2005–10 NHANES surveys show increases in daily energy intake of 180 kcal/day [80] and 108 kcal/day [79] for children aged 2–18 years, respectively. The biggest increase is seen among preschool aged children (aged 2–6 years), whose daily energy intake increased from 1433 kcal/day in 1989–91 to 1664 kcal/day in 2003–06 [75]. Recently, Mendez and colleagues [77] compared energy intake across the 2003–10 NHANES. Results suggest differences in secular trends in energy intake by child age. Daily energy intake decreased between 2003–04 and 2009–10 among children aged 2–5 years (decrease of 220 kcal/day for boys and 132 kcal/day for girls) and 6–11 years (decrease of 230 kcal/day for boys and 142 kcal/day for girls). In contrast, for adolescents aged 12–18 years daily energy intake decreased between the 2003–04 and 2007–08 surveys, but increased between the 2007–08 and 2009–10 surveys (increase of 60 kcal/day for boys and 97 kcal/day for girls).

Changes and patterns in secular-trend surveys should be interpreted cautiously due to a number of limitations, including weaknesses in study design, methodological flaws, different survey methodologies, and random or systematic measurement error in the dietary data [81,82]. For example, the procedural changes between NHANES II and III in dietary survey methodologies, survey food coding, and nutrient composition databases have made comparisons between the two surveys difficult. These limitations could explain inconsistent results in secular-trend surveys.

B Calories From Beverages

The role of caloric beverages in the obesity epidemic has received considerable attention in recent years. In particular, researchers and policymakers have focused on sugar-sweetened beverage (SSB) consumption and its contribution to excess weight gain. SSBs are any beverages that contain added caloric sweeteners, including soda, fruit drinks, sport drinks, low-calorie drinks, and sweetened coffee and tea. National dietary intake data trends indicate SSB consumption by adults and children rose dramatically between 1970s and 2000s, but has decreased in recent years. French and colleagues [83] examined nationally representative data from the 1977–78 Nationwide Food Consumption Survey, 1994–96 Continuing Survey of Food Intake by Individuals, and 1998 Supplemental Children's Survey and found that among children aged 6–17 years, average soft drink consumption increased between 1977–78 and 1998 by 153% for boys and 91% for girls. Similarly, energy intake from SSB increased by

135% between 1977 and 2001 for children and adults [84]. More recently, data suggest decreases in overall energy intake from SSB among youth and adults [85–87]. Among children aged 2–19 years, the mean percentage of daily energy intake from SSB decreased from 13.5% to 10.3% between 2001 and 2010 [85]. Between 1999 and 2010, SSB intake fell for adults aged 20–39 years (from 12.8% to 9.3% of mean daily energy intake) and those aged 60 years and older (from 4.7% to 3.7%), while there were no significant changes for adults aged 40–59 years [87].

There is substantial empirical evidence linking consumption of SSBs to BMI in children and adults [88–91]. The majority of the findings derived from large cross-sectional studies and prospective cohort studies with long periods of follow-up indicate that there is a positive association between SSB intake and unhealthy weight gain and obesity in both children and adults [88,89,91,92]. Moreover, there is emerging evidence from randomized controlled trials that reducing consumption of SSBs may result in reductions in BMI in youth and adults [93–96].

Fruit juice is another caloric beverage that has been in the spotlight for its potential contribution to obesity. The American Heart Association and American Academy of Pediatrics (AAP) recommend limiting daily consumption of 100% fruit juice to 4–6 oz per day for children aged 1–6 years and 8–12 oz per day for children aged 7–18 years [97]. Many young children, however, exceed this recommendation [98–100]. NHANES data from 2007 to 2010 show that 32% of children aged 2–6 years exceed the recommended amount of juice [100]. The mean daily consumption of juice was 9.9 oz for children aged 2–8 years, and 11.2 oz for children aged 9–18 years [100].

There is much debate about the association of 100% fruit juice with obesity [101–103], with mixed findings on the relationship between fruit juice consumption and weight across studies [104]. Some in the field point to the nutritional benefits of juice, the contribution of juice to better diet quality, and the lower cost of fruit juice compared to whole fruit [100,102]. On the other hand, fruit juice consumption in early childhood may be associated with greater consumption of SSBs later in childhood [105] and drinking fruit juice may influence satiety less than consuming whole fruit [106] and contributes to excessive calorie intake [107,108]. The current evidence base and disparate findings makes it difficult to draw conclusions about the association of fruit juice consumption with obesity.

C Eating Away From Home and Fast Food

During the past 40 years, one of the most noticeable changes in eating patterns of Americans has been the increased popularity of eating out. Eating away from home includes foods obtained at restaurants, fast-food places, school cafeterias, and vending machines. The proportion of calories consumed away from home increased between 1965 and 2008 for adults across all income groups, though the percentage of calories eaten away from home is the lowest among low-income adults [109]. Between 1965 and 2008, the percentage of meals eaten or prepared at home decreased by 24.5% for men and 23.9% for women [109]. Frequency of eating away from home did not appear to change significantly after the recession of 2008 [110,111]; rather, data suggest shifts in the type of restaurants frequented, with more Americans choosing fast-food and fast-casual restaurants instead of sit-down restaurants [110–112]. Analysis of cross-sectional data from the American Time Use Study 2003–11 did not indicate changes in rates of eating away from home among adults, even after examining differences by SES [110]. A number of factors account for the increasing trend in eating out, including a growing number of women in the labor force, more two-earner households, a desire for convenience foods because of busy lifestyles and little time for preparing meals, more fast-food outlets offering affordable food, smaller families, and increased advertising and promotion by large food-service chains and fast-food outlets [113].

The trend toward eating away from home more frequently has also been observed among children and adolescents [80,114,115]. Data from the 1977–78 Nationwide Food Consumption Survey, 1989–91 and 1994–98 Continuing Survey of Food Intakes by Individuals, and 2003–06 NHANES for children aged 2–18 years suggest that the percentage of daily energy eaten away from home increased from 23.4% to 33.9% from 1977 to 2006 [80]. Of concern, the study authors also reported that the percentage of energy from fast food increased to surpass food intake in the school setting. By 2007–10, 50% of U.S. children consumed fast food on a typical day [115].

Trends in increased eating away from home may be related to observed increases in energy intake among Americans. Restaurant food is often high in energy, fat, and sodium [116] and food away from home has poorer nutritional quality than food prepared at home [117,118]. Many table-service restaurants provide 1000–2000 calories per meal, amounts equivalent to 35–100% of a full day's energy requirement for most adults [119]. In addition, consumers may view food differently when eating out than when eating at home. Meals or snacks eaten away from home may be seen as an exception to usual dietary patterns; in other words, it may be considered an opportunity to "splurge." Recent estimates from the USDA suggest that for the average adult, eating one meal away from home each week increases daily energy intake by about 134 calories and

translates to approximately two extra pounds each year [117]. Eating out in fast-food restaurants, specifically, has been implicated as a risk factor for obesity. In both youth and adult populations, frequency of fast-food consumption has been positively associated with BMI and obesity [120–123]. In an effort to provide consumers with information about the calories in the foods they are eating, the Food and Drug Administration recently released new guidelines that require calorie information to be posted at the point of sale for many restaurants and fast-food establishments [124].

D Portion Size

Portion sizes, both at home and at restaurants, have become larger over the past several decades [125–127], paralleling increases in obesity prevalence [128–132]. Many restaurants, especially fast-food restaurants, offer large and extra-large portion sizes of products and meals at low cost. The size of a "typical" portion has also increased. For example, Putnam [133] reports that the typical fast-food outlet's hamburger in 1957 contained a little more than 1 oz of cooked meat, compared with up to 6 oz in 1997. Soda pop was 8 oz in 1957, compared with 32–64 oz in 1997. A theater serving of popcorn was 3 cups in 1957, compared with 16 cups (medium size popcorn) in 1997. Analysis of nationally representative data shows that food portion sizes increased between 1977 and 1996 both inside and outside the home [125]. Energy intake and portion size of salty snacks, soft drinks, hamburgers, French fries, and Mexican food increased by 93 kcal, 49 kcal, 97 kcal, 68 kcal, and 133 kcal, respectively, with portion sizes the largest at fast-food restaurants.

Some hypothesize that bigger portion sizes are an important contributor to the obesity epidemic, with observational data supporting this perspective [122,129,130]. [57,58,61] While portion size has not been causally linked to obesity, studies conducted primarily in laboratory settings show that larger portion sizes are associated with greater energy intake [134]. In a meta-analysis of 104 studies, Zlatevska and colleagues [134] found that a 100% increase in portion size resulted in a 39% increase in consumption for adults and a 20% increase for children. Duffey and Popkin [122] examined the role of portion size, frequency of eating, and energy density in relation to change in total energy intake in adults over time. Using data from the Nationwide Food Consumption Survey (1977–78), Continuing Survey of Food Intakes of Individuals (1989–91), and NHANES (1994–98 and 2003–06), the authors found that portion size and number of eating and/or drinking occasions were the strongest predictors of total energy intake. Evidence from randomized controlled trials suggests that limiting portion sizes through portion-control tools (e.g., portion-controlled meals, portion-control plates) can be an effective weight loss strategy [135–137].

E Food Marketing

Although multiple factors influence eating behaviors, one particularly powerful influence is pervasive food marketing. Over the past 50 years, U.S. children and adolescents have increasingly been targeted with intensive and aggressive forms of food marketing and advertising practices [138–142]. Multiple techniques are used to reach children as young as toddlers to foster brand building and influence product purchasing behavior. Content analyses of various types of advertisements indicate that the majority of advertisements are for unhealthy foods [141,143–145]. A recent report found that in 2013, children and adolescents saw in average of 13.1 and 16.5 food advertisements per day on television, respectively [142]. Fast food was the most represented category of food in these advertisements (23% for children and 28% for adolescents), followed by cereal for children (16%) and candy for adolescents (14%). Restaurants (excluding fast food) comprised 12% of advertisements for children and adolescents, ranking third. Food marketing to children now extends beyond television and is expanding rapidly into ever-present and evolving "new media," including social media, apps, advergames, video games, and text messages [146–150].

The 2006 Institute of Medicine report on Food Marketing to Children and Youth concluded that food and beverage marketing practices geared to children and youth are out of balance with recommended healthful diets and contribute to an environment that puts youth's health at risk [151]. The report set forth recommendations to guide the development of effective marketing strategies that promote healthier food, beverages, and meals for children and youth. Among the major recommendations for the food, beverage, and restaurant industries was that the industry should shift their advertising and marketing emphasis to child- and youth-oriented foods and beverages that are healthier. The Children's Food and Beverage Advertising Initiative (CFBAI), a voluntary set of guidelines for marketing toward children, was created in 2006 as an effort in self-regulation by these industries. Despite industry claims (CFBAI 2013 Progress Report, 2014), research indicates that these voluntary guidelines have been largely unsuccessful in shifting the emphasis away from high-energy and low-nutrient foods and beverages to healthful foods and beverages in marketing targeting children [140,152,153]. The CFBAI does not apply to advertising directed at older children (aged 12 years and older), who are also susceptible to advertising and who may be unduly influenced by advertising techniques

that are more difficult to identify, such as social media marketing and product placements [154]. In addition, the CFBAI does not cover many types of child-directed advertising, including product packaging, in-store marketing, video games rated everyone, or PG and PG-13 movies [154].

To improve and limit food marketing viewed by children, experts recommend closing loopholes and expanding the scope of these guidelines [153,155] (Healthy Eating Research, Recommendations for Responsible Food Marketing to Children 2015). If industry-sponsored voluntary guidelines continue to result in only modest improvements in the content and reach of child-directed marketing, Congress should enact legislation mandating reforms. Advocacy and public health groups are also calling on the Federal Trade Commission, the Federal Communications Commission, and Congress to work together with industry to develop comprehensive and clear rules governing the marketing of food and beverages to children—rules that take into account the full spectrum of advertising and marketing practices across all media and apply to all children, including adolescents, as well as that address accountability shortfalls of current efforts [155].

F Eating and Dietary Practices

Much research examining the role of diet in the etiology of obesity has focused on associations between obesity and dietary intake (e.g., energy intake, portion size). As previously discussed, findings from this large body of research leave many questions unanswered. Research on eating practices and behaviors, such as meal patterns, dieting, and binge eating, provides insight on other factors that may influence dietary intake and weight status. In this section, we highlight some of the research that examines associations between specific eating practices and obesity and identify questions that remain unanswered, and make recommendations based on this body of research for future studies.

1 Meal Patterns

Research examining the pattern and timing of eating suggests that the timing and frequency of daily eating occasions may be associated with the development of overweight and obesity, as well as with weight loss [156,157]. Much attention has been paid to the importance of breakfast and the impact skipping this meal on weight gain and weight loss efforts [158–160]. In observational studies, skipping breakfast has been found to be higher among overweight adolescents and adults [159]. In a cross-sectional study of more than 8000 adolescents, usual breakfast consumption was reported by 53% of normal weight youth, 48% of overweight youth (85th–95th percentile), and 43% of obese youth (BMI > 95th percentile) [161]. In addition, several cross-sectional studies report associations between eating breakfast and better nutritional profiles [162] and increased physical activity levels [157] in children and adolescents. Breakfast eaters generally consumed more daily energy, yet were less likely to be overweight [162]. The quality of the breakfast meal is a key consideration, with consumption of ready-to-eat or cooked cereal typically associated with better nutrition and weight outcomes [163,164].

There is, however, relatively little evidence of a causal relationship between eating (or skipping) breakfast and obesity, and randomized trials investigating the impact of skipping breakfast on energy intake and weight loss are limited and report mixed findings [157,158,160]. Because of the cross-sectional nature of the evidence, it is not clear whether breakfast eating or skipping leads to obesity (e.g., it may be associated with higher energy intake at later times in the day) or whether it is a consequence of obesity (e.g., meals are skipped for weight-control purposes). Additional high-quality, randomized studies testing the relationship between breakfast and obesity are needed.

Meal skipping is frequently used as a weight-control method. Among adolescents and adults trying to control their weight, Neumark-Sztainer and colleagues [165] found that skipping meals was commonly reported; 18.6% of adult males and females, 22.8% of adolescent females, and 14.1% of adolescent males trying to control their weight reported skipping meals. An important question relates to the impact of skipping meals on overall energy and diet quality. In a study of women participating in a weight gain prevention study, skipping meals for weight-control purposes was not associated with overall energy intake [166]. However, meal skippers reported higher percentages of total energy intake from fat and from sweets, lower percentages of total energy intake from carbohydrates, and lower fiber intakes than women who did not report meal skipping [166]. Similar to the evidence on skipping breakfast, it is not clear whether meal skipping plays a causal role in the onset of obesity. Additional prospective studies and evaluations of interventions aimed at decreasing meal skipping are needed to assess causality. However, in light of the inverse associations between meal skipping and nutrient intake, and the potential for leading to uncontrolled eating due to hunger, meal skipping should not be recommended as a weight-control strategy. Rather, careful planning of meals with nutrient-dense foods that are low in fat and energy should be encouraged.

Similar to meal skipping, snacking behavior also has an inconsistent relationship with obesity [167,168]. While snacking behavior and the prevalence of obesity increased

simultaneously in the past several decades [74,169], recent studies suggest that relationships between snacking and weight may vary by gender and overweight status [170] and by the types of foods eaten as snacks [170–172]. Chapter 25, Snacking and Energy Balance in Humans, of this volume includes a more detailed discussion of this topic.

2 Dieting Behaviors and Dietary Restraint

Research findings clearly indicate that overweight individuals are more likely to report engaging in dieting and other weight-control behaviors than healthy weight individuals. For example, in a large cross-sectional study of adolescents, dieting behaviors were reported by 17.5% of underweight girls (BMI <15th percentile), 37.9% of healthy weight girls (BMI 15th–85th percentile), 49.3% of overweight girls (BMI 85th–95th percentile), and 52.1% of obese girls (BMI >95th percentile) [173]. In 5- and 10-year follow-ups of the same population, adolescents who reported dieting and unhealthy weight-control behaviors were found to be at increased risk for weight gain and for being overweight over time, even after adjusting for baseline weight status [174,175]. Adolescents who reported dieting and unhealthy weight-control behaviors at baseline had greater increases in BMI over the 10-year period, compared to peers who did not engage in these behaviors at baseline [175]. BMI increases were even greater for those young people who reported persistent dieting and unhealthy weight-control behaviors (i.e., reported at both baseline and the 5-year follow-up). Among persistent dieters, BMI increased by 4.33 for females and 6.96 for males over the 10-year study period, compared to increases of 2.38 and 3.45 among nondieters, respectively. For both females and males, persistent engagement in unhealthy weight-control behaviors predicted greater increases in BMI, compared to participants who did not engage in these behaviors (4.63 vs 2.29 for females and 5.42 vs 3.65 for males, respectively) [175]. Similarly, in a prospective study on adolescent girls by Stice and colleagues [176], baseline dieting behaviors and dietary restraint were found to be associated with obesity onset 4 years later. After controlling for baseline BMI values, the hazard for obesity onset over the 4-year study period was 324% greater for baseline dieters than for baseline nondieters. For each unit increase on the restraint scale, there was a corresponding 192% increase in the hazard for obesity onset [176].

These findings suggest that for some individuals self-reported dieting may be associated with a higher energy intake, and not a lower energy intake as intended. One explanation for this is that self-reported "dieting" may represent a temporary change in eating behaviors, which may be alternated with longer term eating behaviors that are not conducive to weight control. Another explanation is that self-reported dieting and dietary restraint may be associated with increased binge-eating episodes resulting from excessive restraint, control, and hunger. Indeed, Stice and colleagues did report positive, albeit modest, associations between binge eating and both dieting behaviors ($r = 0.20$) and dietary restraint ($r = 0.20$). In an analysis aimed at addressing the somewhat perplexing question as to why dieting leads to weight gain, Neumark-Sztainer and colleagues found that binge eating was an important mediating variable between dieting and weight gain over time in adolescents [174]. Other researchers have also suggested that dietary restraint may lead to binge-eating behaviors [177], thereby placing individuals at risk for weight gain, rather than the intended weight loss or maintenance [178].

3 Binge Eating

Lifetime prevalence of binge-eating disorder in the United States is estimated to be 2.6% [179]. In a cohort of adolescents followed from age 16 to 24 years, 2.3–3.1% of young women and 0.3–1.0% of young men reported binge eating [180]. Overweight individuals are more likely to engage in binge-eating behaviors than their normal weight counterparts among youth and adults [181–183]. In a nonclinical sample of adult women enrolled in a weight gain prevention program, binge eating was reported by 9% of normal weight women and 21% of overweight women [181]. Furthermore, binge eating tends to be more prevalent among overweight individuals seeking treatment for weight loss. It is estimated that about 30% of participants seeking weight loss counseling engage in binge eating [184], with about 3.5–5% meeting clinical criteria for Binge-Eating Disorder, the majority of whom are overweight or obese [185,186].

Success in weight loss efforts may be influenced by binge-eating behaviors, though previous studies report mixed findings. While most studies show that binge eating at the commencement of weight loss treatment does not influence weight loss success [187–191], several studies have found that baseline binge-eating status is related to program compliance and attrition [189,190] and may be related to weight loss maintenance [192–194]. Previous research has found that behavioral weight loss programs may positively impact binge-eating behaviors [193,195,196], with improvements in binge eating associated with better weight loss outcomes and maintenance [192,195–197]. In a weight loss maintenance study of adult women and men who had previously lost at least 10% of their body weight, prevalence of binge eating at any point during the study period was 30.1% [192]. Results from this study suggest that consistency of binge

eating may be important to weight loss maintenance; binge eating at baseline was not associated with weight regain, but those adults who reported binge eating during the study period had a higher rate of weight regain than those who did not report binge eating at any time point.

In working with overweight individuals within health care and other settings, it is essential to be sensitive to the daily struggles overweight clients may face within thin-oriented societies. Risk factors for binge eating include the internalization of negative weight stereotypes and thin-ideals, perceived pressure to be thin, and body dissatisfaction [198–201]. Overweight clients may be reluctant to share their binge-eating experiences; therefore a nonjudgmental attitude on the part of the health care provider is critical. For some individuals, hunger resulting from dieting or meal skipping may be a cause of binge eating, while for others binge eating may be a response to stress. Some individuals may be experiencing cyclical patterns; for example, emotional stress leads to binge eating, which leads to further emotional stress, which leads to further binge eating. Strategies for avoiding binge eating should be linked to factors that appear to be leading to binge eating for each individual.

4 Family Influences on Dietary Intake and Eating Practices

A considerable amount of research has been devoted to the role of the family in the etiology, prevention, and treatment of childhood obesity [202–204]. Family-based obesity treatments are rooted in the premise that the home environment and parenting practices are critical to the eating and activity behavior changes needed to successfully promote and sustain healthful body weight for children [205–207]. Epstein's parent-family-based obesity treatment produced impressive results. At the 10-year follow-up, 33% of all children decreased their BMI by 20% or more from baseline, and 30% were no longer obese. Other work supports the idea of parent-focused child-obesity treatment. After 7 years of follow-up, children whose parents received a parent-targeted intervention were significantly less likely to be obese compared to children who were themselves the target of the initial intervention (30% and 69%, respectively) [207]. These results suggest that targeting parents is of critical importance.

With regard to dietary intake and eating practices, questions arise as to how the family environment influences individual family members' eating behaviors and what parents and family can do to support healthy eating behaviors. The aim is to provide an environment in which healthful food is available, eaten in an enjoyable manner, and consumed in appropriate amounts. Most of the research in this area has focused on the influence of parents on their children's eating behaviors. Parents/caretakers may influence their children's dietary intake and eating practices via numerous channels. Some of the key channels include the home food environment [208–211] (including food availability, preparation, and accessibility), family meal patterns [212], infant and child feeding practices [213,214], and role modeling of eating behaviors and body image attitudes [215]. Research in the arena of adolescent health indicates that general family context variables are strongly associated with the emotional well-being of adolescents and with eating and other health-related behaviors [216].

The role of parenting in the promotion of healthy eating behavior and activity patterns has received increased attention in recent years. Research has focused on the role of domain-specific parenting styles, such as parent feeding practices, as well as the role of more general parenting styles and practices, such as authoritative versus permissive parenting. Food-specific parenting practices have been shown to be related to eating patterns, with some practices linked to healthy eating behaviors and others to unhealthy eating behaviors [217–220]. For example, restrictive feeding practices have been found to be associated with decreased fruit and vegetable intake [221] and greater child interest and desire for restricted foods [222], suggesting this may be a counterproductive strategy. For general parenting styles, the literature is more mixed; some studies do not support an association between parenting style and child dietary intake, while others have found that certain parenting styles, particularly authoritative parenting, are associated with healthier eating patterns [223]. Further research is needed on the relationship between general parenting styles and child dietary intake to provide a better understanding of how and through what mechanisms general parenting may affect child eating patterns. There is significant evidence of an association between family meals and child and adolescent dietary intake [212,224]. Previous research on children and adolescents has found that eating together with family is associated with greater intake of healthy food and lower intake of unhealthy food [212]. There is, however, inconsistent evidence linking family meals to child and adolescent weight status. Most research to date has been cross-sectional; several studies have found no association between family meals and child and adolescent BMI, while others have found that greater frequency of family meals is inversely associated with child BMI [225]. A recent longitudinal analysis provides evidence of a relationship between family meals during adolescence and weight status as a young adult. Berge and colleagues [226] examined 10-year weight outcomes of adolescents enrolled in Project EAT I and Project EAT III and found that those participants who reported having family meals at baseline had lower odds of overweight or obesity in

young adulthood, compared to those participants who reported no family meals. The association was strongest among African American participants, though results were significant among all racial and ethnic groups.

Although there is ongoing work in this area, research to date suggests that providing a healthy home food environment and healthy routines, such as family meals, are important. This allows children to make choices within their environment regarding exactly what and how much to eat and allowing for the development of internal regulation of food intake.

IV PHYSICAL ACTIVITY

Prominent among the health benefits associated with a physically active lifestyle is the protective effect of physical activity on obesity. Cross-sectional research shows that lighter individuals are more active than heavier individuals and prospective research indicates that changes in physical activity level are associated with changes in body weight in the direction predicted by the energy balance equation [227–230]. The majority of studies conducted in children also find that physical activity levels and body weight are negatively associated [231–233]. Exercise has also been shown to improve short- and long-term weight loss in experimental studies in both children and adults [234–236] and is a key factor in successful weight loss maintenance [237–239]. Despite these relationships, the weight losses achieved through changes in physical activity alone are often minimal. Therefore, recommendations for weight control include changes to both diet and physical activity [239].

A Prevalence of Leisure-Time Physical Activity in Adults

The 2008 United States Department of Health & Human Services Physical Activity Guidelines recommend that adults engage in 150 minutes a week of moderate-intensity, or 75 minutes a week of vigorous-intensity aerobic physical activity (or an equivalent combination of moderate- and vigorous-intensity aerobic physical activity) to obtain substantial health benefits. An approximate doubling of these amounts is recommended to obtain additional health benefits and two or more weekly muscle-strengthening activities are recommended in addition to aerobic activity. In order to achieve weight loss and weight maintenance, higher levels of moderate-to-vigorous physical activity (MVPA) (150–250 minutes per week) are recommended [239].

Despite the benefits of physical activity for body weight regulation and health, many individuals in the United States are insufficiently active or inactive. Using self-reported measures of physical activity, data from the BRFSS survey indicated that only 20.6% of American adults met guidelines for physical activity in 2011 [240]. Similar to prevalence with overweight and obesity, there are differences in physical activity by sex, race/ethnicity, age, and education: men tend to be more active than women; younger adults are more likely to be active than older adults, with the lowest levels of activity observed among those 65 years and older, and non-Hispanic whites report the least leisure-time physical activity compared to other racial and/or ethnic groups [240,241]. Socioeconomic disparities in physical activity are also observed, with education and income positively associated with leisure-time physical activity level [242,243]. However, when considering total physical activity (including household and occupational activity), many of these disparities are decreased or reversed [244].

B Prevalence of Leisure-Time Physical Activity in Youth

Although estimates of physical activity levels among youth tend to be higher than self-reports by adults, the prevalence of regular physical activity is still surprisingly low. Current physical activity guidelines for children suggest that children should get 60 minutes of activity daily which should include muscle-strengthening activities at least 3 days per week and 3 days bone strengthening activities [245]. Parent-reported data from the NHANES suggest that 70% of children aged 9–11 were meeting these recommendations in 2009 [246]. The availability of data on physical activity patterns and prevalence varies as a function of child age group. Representative survey data on the physical activity patterns of young children are not available, in part because of methodological difficulties in collecting such data from children. Young children are limited in their ability to accurately recall their activity patterns, and the unplanned, unstructured nature of children's physical activity patterns does not lend itself well to the self-report format employed in large-scale surveys. Increasingly, accelerometry is used to estimate children's physical activity levels, with Actigraphs used to measure physical activity in NHANES beginning in 2003.

According to data from the CDC's Youth Media Campaign Longitudinal Survey (YMCLS), a nationally representative survey of children aged 9–13 years and their parents was conducted between 2002 and 2006 [247,248]. Data from the 2002 survey found that 61.5% of children aged 9–13 years did not participate in any organized physical activity during their nonschool hours and that 22.6% did not engage in any free-time physical activity. Between 2002 and 2006, physical activity levels reported on the YMCLS remained stable or slightly increased. Examination of 2003–04

and 2005–06 NHANES physical activity data measured by accelerometry among 6- to 19-year olds showed that 6- to 11-year olds spent over twice as much more time (88 minutes per day) engaged in MVPA compared to 12- to 15-year olds and 16- to 19-year olds who engaged in 33 minutes/day and 26 minutes/day, respectively [249]. Data from the Youth Risk Behavior Surveillance Surveys (YRBS) among U.S. high school students showed that in 2007, just over one-third of youth reported any kind of physical activity that increased their heart rate and made them breathe hard some of the time for a total of at least 60 minutes per day on 5 of the 7 days preceding the survey [250]. The 2007 YRBS data also showed that just under one-third of adolescents participated in insufficient amounts of physical activity. Similar to patterns observed among adults, across multiple national surveys, physical activity levels tend to be higher among boys compared to girls and higher among non-Hispanic White youth compared to Black and Hispanic youth [251]. Moreover, activity levels predictably decline as children transit into adolescence.

C Determinants of Physical Activity

In addition to the demographic determinants of physical activity, including age, gender, race/ethnicity, etc., there are important modifiable determinates of physical activity that can be leveraged to enhance physical activity and weight-control interventions. Although there are numerous potential determinants to consider (e.g., see Ref. [252]), a selection of impactful determinants will be presented here for consideration.

1 Self-Efficacy

Self-efficacy is an individual's belief in his or her ability to successfully engage in a given behavior which may influence the activities that individuals choose to approach, the effort expended on such activities, and the degree of persistence demonstrated in the face of failure or aversive stimuli [253]. Exercise self-efficacy is the degree of confidence an individual has in his or her ability to be physically active under a number of specific/different circumstances or, in other words, efficacy to overcome barriers to exercise [254]. Exercise self-efficacy is one of the strongest and most consistent predictors of exercise behavior in adults [255–260]. Self-efficacy is particularly important during challenging transitions such as beginning a physical activity routine [257] or when faced with barriers to remaining active [260]. Self-efficacy has also been shown to be highly related to physical activity in youth [261–264]. Perceived competency, an aspect of self-efficacy, is predictive of maintaining activity during the transition from adolescence to adulthood, specifically among females [265].

2 Social Support

Social support is another strong correlate of physical activity for both youth [266–275] and adults [276–283]. Adults who engage in regular exercise report more support for activity from people in their home, work, and social environments. Instrumental support may be particularly important for initiation efforts among sedentary adults [284]. Carron et al. [285] examined six major sources of social influence on physical activity, including important others such as physicians or work colleagues, family member, exercise instructors, or other in-class professionals, co-exercisers, and members of exercise groups, in a comprehensive review. In studies testing how social support may influence physical activity, social support has been found to influence self-regulation behaviors associated with higher levels of physical activity, both directly and indirectly, through self-efficacy [286] and through motivational variables [287]. Further investigation of the pathways through which social support influences physical activity is important for developing effective interventions that capitalize on social influences.

Positive family environments and family support for physical activity is a robust correlate of physical activity for both boys and girls [267,269–272,274,275]. A comprehensive review examining the relationship between parental social support and youth physical activity identified two overarching categories of support, tangible and intangible [288]. Within these overarching categories, subcategories were identified. Tangible support included instrumental support, such as purchasing equipment, payment of fees, and transportation and conditional support which included either involvement of the parent in the activity or being physically present, but not directly participating in the activity. Intangible support included motivational support such as providing encouragement and praise, and informational support. Across the 80 reviewed studies, the majority demonstrated positive associations between the various types of parental support and physical activity. Peer support has also been shown to be a strong correlate of youth physical activity level with the influence of peers increasing over time [267,268,273,289].

The use of online social networks has grown in recent years: currently 65% of American adults regularly use online social networks [290]. With this growth, there is also growing acknowledgment of the role online social support can play in supporting health behaviors such as physical activity. While this is a newer area of research, there is evidence that individuals who use online social networks seek and gain social support for physical

activity and other health behaviors [291,292]. These platforms can be harnessed to enhance social support during intervention (e.g., see Ref. [293]) and may be especially useful when intervening with individuals whose unique social support needs may not be fulfilled through traditional social support networks. As an example, online social support was utilized to help improve physical behaviors of young adult cancer survivors, a group for whom social support is vital but difficult to create through traditional means [294].

3 Overweight and/or Discomfort With Physical Activity

Clearly, body weight and physical activity are inextricably linked. Although it is clear that increasing physical activity is an important factor in regulating body weight, weight status may serve as a barrier to physical activity. This is due in part to physical activity being less pleasurable (e.g., it is uncomfortable for people to exercise when they are heavier), and in part because of embarrassment (e.g., individuals report feeling embarrassed about being seen in public in exercise clothes, at gyms, due to weight status and societal reactions toward overweight individuals) [295] or due to body dissatisfaction [296]. However, weight status can also be a motivator for initiating exercise. One of the most common reasons adults give for exercising is weight control, and physical activity is one of the strongest correlates of successful weight loss and maintenance [297]. Physical activity promotion programs, however, need to be modified to address the needs of overweight youth and adults. Longitudinal data on adolescents indicate that body dissatisfaction predicts lower levels of physical activity, suggesting a need for physical activity interventions that help individuals feel more comfortable with their bodies, regardless of their weight status [298].

4 Exercise History

Prior history of physical activity should positively influence future physical activity behavior by promoting and shaping self-efficacy for exercise and by developing physical activity skills. The observed relationship between exercise history and exercise behavior varies, however, depending on how exercise history is defined and the time period over which physical activity behavior is assessed. Physical activity has been shown to track moderately well across childhood and adolescence [299] and in adulthood [300]. A systematic review of 27 papers from 16 different cohorts that included baseline age ranges from 8 to 10 years, with follow-up duration ranging in length from 5 to 55 years suggested that physical activity tends to track more strongly in boys compared to girls, at older ages, and tracks less strongly with increasing years of follow-up [301]. Youth who participate in sports tend to be more likely to be physically active as young adults [302,303]. Interestingly, there is an emerging literature suggesting that sedentary activities such as television viewing and video game use also track across childhood [304]. The perception of the exercise experience as a child may be as important as amount of childhood exercise. One recent study found that recalling being forced to exercise as a child was associated with lower levels of physical activity in adulthood [305]. A child's enjoyment of physical activity [306,307] and enjoyment of physical education experiences [308] are significant predictors of physical activity levels. Creating positive environments for physical activity for youth is likely a key factor in promoting higher levels of physical activity as a lifestyle habit.

5 Time

Among adults, time constraints are the most frequent barriers to exercise and are reported by both sedentary and active individuals across diverse populations with respect to age, race/ethnicity, and gender [309–315]. Even among regular exercisers, scheduling efficacy remains an important and significant predictor of adherence [254]. Therefore, to maintain exercise adherence, regular exercisers have to become adept at dealing with time as a barrier. The time barrier may be a particular problem for certain population subgroups. For example, time spent caring for children may make it difficult for parents to maintain a regular physical activity program [315].

Several physical activity intervention approaches geared toward addressing the time barrier have been developed in recent years. These include strategies to help people fit exercise and physical activity into their lives without necessarily having to engage in center- or gym-based activities, such as home-based programs, using either phone- [316–318], mail- [319,320], or technology-based [319,321–323] delivery modalities (e.g., web-based options, smart phones). Another strategy to address the time barrier has been to focus on the potential health benefits of integrating multiple short bouts (<10 minutes) of physical activity across the day. A recent systematic review of the evidence for the effectiveness of short activity bouts that are incorporated into organizational routines in schools and work settings concluded that interventions that integrate physical activity into organizational routines have shown modest but consistent benefits [324]. Even shorter bouts of high-intensity training of <5 minutes have received growing attention and is a potential intervention approach to offset the perception of limited time [325].

6 Access and Environmental Factors and the "Built Environment"

Another barrier that has received increasing attention in recent years is access to exercise facilities, including parks and recreational facilities and safe and attractive places to walk and play outside. The burgeoning literature on the linkages between the built environment and physical inactivity and obesity has increasingly recognized the complexity in measuring the various aspects of the environment that are thought to be important. Brownson and colleagues [326] categorize measurement of the built environment in the following three ways: (1) perceived measures obtained by telephone interview or self-administered questionnaires; (2) observational measures obtained using systematic observational methods (audits); and (3) archival data sets that are often layered and analyzed with geographic information systems. An extensive review of this literature is beyond the scope of this chapter; however, recent comprehensive reviews are available [327]. Distance between individuals' homes and recreational and exercise facilities and/or density of such facilities has been shown to be negatively correlated with exercise behavior in adults [328]. Depending on an individual's activity preference, access to exercise facilities may or may not be related to exercise levels. For those individuals who prefer exercises such as walking or running, which can be done anywhere, access to facilities may be less relevant. Additionally, for those who exercise with home equipment, which could include stationary bikes, treadmills, and even exercise videos, access to facilities may also not affect exercise adherence. Regardless, the extent to which environments are conducive to physical activity likely has a strong impact on population activity levels (e.g., walking/biking paths, safe streets). In addition to objective measures of availability, perceived availability and perceived safety are important [327].

Physical activity among youth appears to be particularly strongly influenced by environmental factors [327,329,330]. The amount of time children spend playing outside has been shown to be a strong correlate of physical activity levels in some [331,332], but not all [333]. Children who live in neighborhoods where play spaces are not adequate are going to have more difficulty achieving recommended levels of physical activity. Further, both child and parent perceptions of neighborhood safety influence children's levels of physical activity [334]. Inequality in availability of physical activity facilities and access to safe play spaces may contribute to ethnic and socioeconomic disparities in physical activity and overweight patterns among youth. Use of after-school time for sports and physical activity, access to community sports activities, and frequency of parents transporting children to activity locations have all been shown to be correlates of physical activity in boys and girls [335–339]. The extent to which families have time and resources to support their children in physical activity pursuits will also have a strong impact on children's activity levels. Anecdotal reports suggest that children spend less time in unstructured physical activities (e.g., neighborhood pick-up games, hide and seek, tag) than in previous years. In contrast, there appears to have been an increase in community-organized sports (e.g., traveling soccer and basketball teams) that require increased parental time, involvement, and financial resources. These factors may potentially contribute to decreases in physical activity and increased socioeconomic differences in physical activity and obesity risk among youth and is an area worthy of further exploration.

a Sedentary Behavior

A related but separate concern from physical activity is the rise of sedentary behavior. Although low levels of leisure-time physical activity likely contribute to the epidemic of obesity, leisure-time activity has remained stable or increased since the mid-1980s, the period during which the prevalence of obesity increased [340]. The past century, however, machines with motors have replaced human labor in virtually every aspect of life, so that the energy expenditure now required for daily life is a fraction of what it was a generation or two ago. Recent examination of data from the U.S. Bureau of Labor Statistics and NHANES estimates that over the last 50 years occupation-related energy expenditure has decreased by more than 100 calories per day [341]. Simultaneous with this change, technology has increased opportunities for sedentary leisure-time activities as well, including television, personal computer use, and mobile devices. This combined change has led to an increase in sedentary activity. Objectively assessed data from NHANES suggest that American adults are sedentary 7.7 hours per day [342] and children are sedentary for 5.5–8.5 hours [251]. Sedentary behavior has recently received growing attention as a separate risk factor for mortality above and beyond physical activity [343].

Television viewing is a major source of inactivity and has received considerable attention as a risk factor for obesity. In addition to contributing to sedentary behavior and displacing time potentially spent in more active pursuits, television viewing has been hypothesized to contribute to excess energy intake [344] and can serve as a cue for eating high-fat, high-energy foods [345]. According to data provided by A.C. Nielsen Company, the average household television set is turned on for more than 7 hours per day [346]. Survey data estimating the frequency of television watching are necessary, however, because television viewing is not necessarily the primary

activity when the television set is turned on. Data from the Americans' Use of Time study show that free time spent watching television increased from about 10.4 hours per week in 1965 to about 18.9 hours in 2010 [347]. Nielsen data indicate that the amount of time per day adults spend watching television is closer to 5 hours per day. Increasingly, adults are watching "time shifted" television and simultaneously watching television while using the Internet.

The high frequency of television viewing and other "screen time" among youth is disturbing. A recent review of the literature indicates that boys and girls are watching 1.5−3.7 hours and 1.4−3.0 hours of television per day, respectively [348]. The AAP recommends that children over the age of 2 watch no more than 2 hours of "screen time" per day. Recent examination of NHANES data (2001−06) indicated that nearly half (47%) of the children and adolescents spent ≥2 hours per day total screen time, largely driven by time spent viewing television [349]. Examination of media use data in younger children, including toddlers and infants, is also concerning. Despite the fact that the AAP discourages media use and screen time for children under the age of 2, a telephone survey conducted in Washington and Minnesota indicated that about 40% of children regularly watched television, DVDs, or videos by 3 months of age, with about 90% of children regularly experiencing screen time by 24 months of age [350]. Examination of data from the National Longitudinal Survey of Youth (1990−98) indicated that almost one-fifth of 0- to 11-month olds and almost half of 12- to 23-months olds watched more television than the AAP recommends [351]. Demographic factors associated with higher screen time in younger children include lower levels of parent education [351] and race/ethnicity with African American and Hispanic children tend to watch more television than non-Hispanic White children [351,352].

Cross-sectional research has shown that there is a consistently strong positive relationship between television watching and obesity in children [333,345,353−358] and adults [359−363]. Although it has been hypothesized that television watching influences obesity by replacing time that could otherwise be spent engaging in more active pursuits, data suggest that this relationship may not always be so simple (i.e., increases in sedentary behavior may result in decreases in physical activity, but physical activity does not necessarily increase in the context of reductions in sedentary activity) [364] and that physical activity and television viewing are often independent predictors of obesity [353]. Additional hypotheses regarding associations between television watching and obesity implicate increases in energy intake linked with television watching. The 2005 Institute of Medicine report focused on food marketing to children concluded that there is strong evidence that television advertising influences children's food preferences and requests, short-term food consumption patterns, and possibly usual dietary intake, and that exposure to advertising is associated with adiposity in children [365].

V CONCLUSION

We have reviewed the literature on key diet and physical activity related risk factors for obesity in children and adults. Highlights related to dietary intake include (1) recognizing the contribution of total energy intake to energy regulation; (2) the influence of eating practices such as eating out, breakfast skipping, restrained eating, and binge eating on obesity; and (3) social and environmental factors that promote excess energy intake. Highlights of the review from the physical activity domain include (1) the importance of addressing the influence of both leisure-time physical activity and sedentary behavior to total energy expenditure; (2) the importance of fostering both self-efficacy and social support for physical activity to promote higher levels of physical activity; and (3) the influence of environmental factors on physical activity levels. To effectively combat the public health problem of obesity, interventions and policies that target change in dietary intake and physical activity are necessary. Intervention efforts must take into account that dietary intake and physical activity are complex, multidetermined behaviors influenced by individual, social, and environmental factors [366].

The etiology of obesity is complex and encompasses a wide variety of social, behavioral, cultural, environmental, physiological, and genetic factors. To effectively address the obesity epidemic in the United States, considerable effort must be focused on helping individuals at the population level to modify their diets and increase their physical activity levels, key behaviors involved in the regulation of body weight. Educational and environmental interventions that support diet and exercise patterns associated with healthy body weight must be developed and evaluated. Prevention of obesity should begin early in life and involve the development and maintenance of healthy eating and physical activity patterns. These patterns need to be reinforced at home, in schools, and throughout the community. Public health agencies, communities, government, health organizations, the media, and the food and health industry must form alliances if we are to combat obesity.

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Chapter 25

Snacking and Energy Balance in Humans

V. Drapeau, S. Pomerleau and V. Provencher
Laval University, Quebec, QC, Canada

I INTRODUCTION

Increasing rates of overweight and obesity over the past decades have coincided with changes in dietary patterns. Snacks have become a major source of the daily energy intake in the population. Despite the vast body of existing evidence studying the impact of snacking on energy balance in humans, it remains unclear whether snacking contributes to overweight or whether it can help with weight management.

Although this chapter reviewed studies involving adults and children, it is not a systematic review of the literature. It first presents the current definitions that describe snacking and how these definitions influence results observed throughout the literature. The prevalence of snacking and the types of food consumed as snacks are detailed in order to get a broader picture of the situation. Thereafter, evidence showing the effects of snacking on energy balance is presented and highlights both the beneficial/neutral and the negative impacts of snacking on energy balance. For a better understanding of why snacking may be seen as beneficial or as a risk factor, possible mechanisms are also explained. At the end, some recommendations are presented on how snacks can be integrated into a healthy diet.

II DEFINITION OF SNACKING

The impact of snacking on energy balance is a significant source of controversy in the literature. Snacking has been recognized to have positive outcomes such as the contribution of nutrients to the diet, lower body mass index (BMI) and greater weight loss, but it has also been associated with negative outcomes such as weight gain and obesity. Defining snacking is not a simple task as it includes different dimensions such as "what," "how much," "when," and "how" foods are eaten and is influenced by cultural norms. These differences likely explain why no consistent definition has been adopted among researchers in the field (Fig. 25.1).

The different definitions of snacking used in the literature have been recognized as the principal factors influencing the link between snacking and energy balance. Many definitions have been proposed, which all have strengths and weaknesses. The most common definitions are based on (1) time of day food is consumed, (2) portion size, (3) time and portion size, (4) number of eating events per day, (5) self-reporting, (6) type of food and its nutrient profile, (7) where eating occurs, and (8) physiological responses. The first four criteria—which seem to be favored in clinical trials—can be objectively defined and measured. The subsequent three are more subjective and frequently employed in large research surveys in which collecting detailed snacking data may not be feasible. Although often used interchangeably, these definitions address different dimensions of snacking with some overlap between them. Besides these definitions based on observations and reporting of eating behaviors, another interesting definition founded on physiological responses was proposed. This definition is related to the presence or absence of a triggering physiological stimulus. However, this method has been less commonly used so far. Thus, at this time, it is critical that the strengths and weaknesses of different definitions be understood so that appropriate inferences can be drawn from the data collected from each. Moreover, since no single definition adequately covers the different dimensions, these definitions should be used to derive a global definition. Table 25.1 presents the different definitions with their strengths and principal limitations.

In summary, there is no single, widely accepted definition of snacking. Each approach holds advantages and inconveniences. It is also noteworthy that most of these

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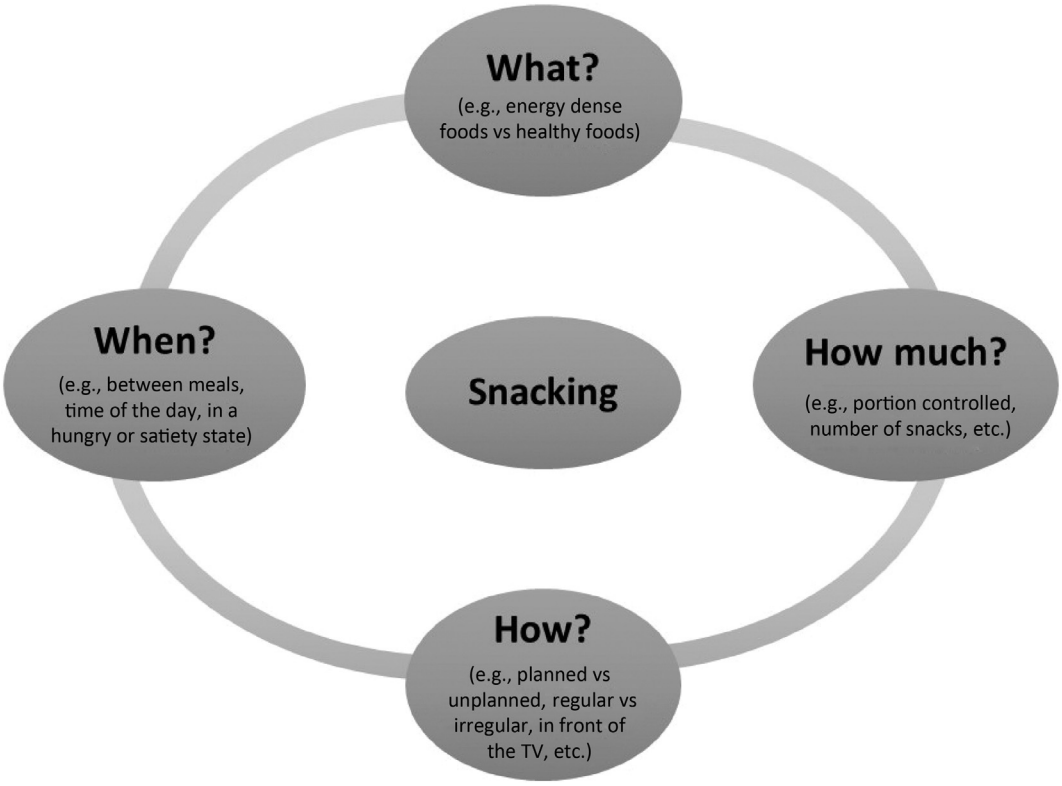

FIGURE 25.1 Dimensions of snacking patterns. *Adapted from C. Berg, H.B. Forslund, The influence of portion size and timing of meals on weight balance and obesity, Curr. Obes. Rep. 4 (2015) 11–18 [1].*

definitions are subject to underreporting as they are usually assessed with 24-hour dietary recalls, food diaries, or food frequency questionnaires. This limitation will be further discussed in another section. The selection of a definition depends on the study objectives and the feasibility of collecting different types of information. It is important to note that findings based on one definition are difficult to interpret and to compare with other results not using the same definition. Another critical point—there is no single measure that is broadly representative of all snacking dimensions. Perhaps, the combination of more than one definition could represent an avenue to adequately assess snacking behaviors.

III PREVALENCE OF SNACKING

In the United States, data from National Health and Nutrition Examination Survey (NHANES) showed that snacking behaviors have changed over the last four decades [3]. In comparison to the 1971–74 surveys, results from 2007 to 2010 revealed a greater proportion of adults eating snacks. Overall, 90% of the population consumed snacks in both genders. These results are based on participant self-reporting where snack was defined as "any eating event identified as snack, drink, between meals, extended consumption." Furthermore, data from 2007 to 2010 indicated that approximately 66% of US adults consumed two or more snacks per day compared to 63% in 1971–74. Consequently, the percentage of people consuming more than 50% of daily energy intakes from snacks has significantly increased among men and women. Interestingly, while the interval between breakfast and lunch remained unchanged, the interval between lunch and dinner was slightly longer, which has resulted in a shorter interval between dinner and after-dinner snacks over the decades.

Similar results have also been observed for adults in Europe (France [4], United Kingdom [5], and Finland [6]) and in Latin America (Brazil [5] and Mexico [7]). While trends showing higher rates of snack consumption over the last years have also been noted in China, the proportion of snackers remains smaller (37% of adults consuming snacks) than other countries [8]. Although cultural differences may be seen among countries (e.g., number and times of daily eating episodes), trends showing an increase of eating patterns that include snacks remain constant around the world [9]. Incidentally, a "snack-dominated eating pattern" has been described among Finnish population representing the habit of consuming a majority of daily energy from snacks [5]. A similar pattern was

TABLE 25.1 Different Snacking Definitions

Snacking Dimension	Definition	Strengths	Limitations
1. Time of the day	Based on the consumption of foods at specific times of day (mid-morning, mid-afternoon, and evening)	Convenient for data analysis by avoiding ambiguous or arbitrary food attribute quantification such as form, nutrient profile, or portion size	Subject to error due to different lifestyles and eating patterns, for example, cross-culturally, the main eating event in the North America tends to occur at approximately 6 p.m. versus 10 p.m. in some European countries; eating early or late in response to working schedule may shift meals to snacks and vice versa
2. Portion size	Based on the consumption of a snack with a portion size smaller than that of a meal. Can be calculated by summing the calories from two or more foods consumed together	Avoid limitations attributable to defining what time of day a snack or meal occurs	Still requires an arbitrary criterion for differentiating meals from snacks; Difficult to assess cultural practices; Subject to time trends, that is, portion size of snacks may increase with rising daily energy intakes and portion sizes complicating the interpretation of findings from the literature
3. Time and portion size	Based on foods or a group of foods, with a predetermined portion size, consumed after a specific period of time following other eating events with a defined portion size	More objectively measured data because it considers the intervals between two eating occasions and the energy content of the foods consumed within an eating event	Based on arbitrary criteria defined by the researcher versus observed cultural patterns
			Not applicable for large surveys because the necessary level of time and portion size detail is often not available (e.g., with food frequency questionnaires)
4. Number of eating events	Based on the number of eating events which usually includes regular meals and snacking	Provides more flexibility than the "time of day" definition because meals do not have to be eaten within specific times of the day	Eating events usually self-defined by the respondents, which can result in different definitions applied across individuals or cultures, hampering comparative analyses
	Meals are expected to follow a relatively fixed routine, especially during the week (breakfast, lunch, and dinner), whereas snacking is assumed to be less structured and regular and thus may occur at different times and places	Can be used for individuals with different lifestyles and cultural backgrounds	Requires a researcher-imposed set of criteria, and no consensus on metrics exists
5. Self-reporting	Based on self-reported foods that are consumed as part of a meal or snack	Commonly used approach in epidemiological studies	Based on self-reported data which can be significantly affected by cultural norms and perceptions, for example, if the participant criterion to identify a snack was based on food type or portion size, it is unclear how this respondent would report the same food eaten as a meal or how another participant would classify the same food as a snack
		Has strong cultural connotations but largely defies objective documentation	

(*Continued*)

TABLE 25.1 (Continued)

Snacking Dimension	Definition	Strengths	Limitations
6. Types of food and nutrient profiles	Based on the nutrient profile of foods snacks can be classified according to nutrient density; several classification systems identifying high-quality snacks exist, but criteria vary, with little congruence between systems	Could help to identify specific foods associated with better outcomes on energy balance and health	Subject to misclassification of snack food items. Some foods have mixed profiles providing important nutrients and high energy density (e.g., ice cream) and/or can have other properties that do not promote positive energy balance or weight gain (e.g., nuts)
7. Where snacking occurs	Based on the place where snacking occurs. In this case, meals are assumed to be eaten at a fixed place such as a table at home or at a restaurant, whereas snack foods are, by default, consumed elsewhere	Avoid issues related to food type, nutrient content, time of day, and portion size and is easily distinguished from a meal	Cannot easily be distinguished from secondary eating and drinking, that is, when individuals indicate another activity as their primary focus, for example, eating while driving, etc.
8. Physiological response	Based on the presence or absence of a triggering physiological stimulus, that is, drop in blood glucose, a snack would be defined as an eating event not triggered by hunger, but instead elicited by an external nonphysiological stimulus	Objective measure	Does not consider time of consumption or nutrient profile Does not consider differences in metabolic status before a meal or a snack, which can influence substrate oxidation

Source: Adapted from R. Mattes, S.Y. Tan, Snacking and energy balance in humans (Chapter 27), in: A. Coulston, C. Boushey, M. Ferruzzi (Eds.), Nutrition in the Prevention and Treatment of Disease, third ed., Academic Press, New York, NY, 2013 [2].

noted in Brazil where nearly 23% of the population reported eating more than three snacks per day (defined as heavy snackers) [6]. The average number of snacks reported was 1.6 snacks per day. This average was nearly the same in Mexico [7] while it was a little over two snack episodes per day in the United States [3,10].

Nationally representative survey data indicated that the contribution of snacks to the overall dietary intake of U.S. children and adolescents also increased over the past few decades [11,12]. The prevalence of snacking, that is, at least one snack over two recall days, increased from 74% in 1977–78 to 98% in 2003–06 [11]. Over the same period, the average frequency of snacking increased by one occasion per day to reach an average of two snacking occasions per day in 2003–06 and the contribution of snacking to total energy intake increased to 27% in 2003–06. Analyses of trends in dietary intake in U.S. children have also shown that there was a significant increase in the contribution of energy-dense foods, nutrient poor foods, and beverages to the total daily energy over the past decades [13].

Overall, snacks represent roughly 20–25% of daily energy intake for both adults and children [3,7,11,14,15]. In Canada, snacks now provide more calories than breakfast [14]. American data from 2003 to 2006 showed an increase in the daily energy intake coming from snacks for children (from 244 to 496 kcal/day) and adults (from 196 to 472 kcal/day) when compared to data from 1977 to 1978 [16]. Energy per snacks has also risen from 502 to 634 kcal/snack among men and from 296 to 438 kcal/snack among women in the United States over the last 40 years [3]. In Norway, energy content per snack was slightly lower with 406 and 430 kcal for women and men, respectively [15] and in Mexico, total energy per snack was even lower with an average of 178 kcal for both genders and all ages [7]. In Brazil, light snackers (one to two snacks per day) consumed more calories per snack when compared with heavy snackers (280 vs 233 kcal, respectively) [6]. However, heavy snackers consumed almost twice as many calories per day from snacks compared with light snackers (832 vs 422 kcal, respectively). In the case of the heavy snackers, energy from snacks accounted for 35.5% of the daily energy intake, while it was 21.7% for light snackers. Among Brazilians, the largest number of snacks was consumed in the afternoon or early evening whereas the largest volume (grams/snack) and energy content per snack were reported in the late morning and late at night [6]. In China, the most popular time for taking a snack was the evening [8] while it was mid-morning and mid-afternoon in Mexico [7]. It should be noted that

in Great Britain, snacking peaks at three moments during the day, which are usually 2−3 hours after breakfast, lunch, and dinner [17]. These data show cultural differences that are mostly explained by the time of the day meals are consumed and, consequently, may affect the timing of snacks [9].

IV SNACKING AND TYPE OF FOOD CONSUMED

For both adults and children, compared with foods eaten at meals, foods consumed as snacks are often lower in fat and protein while being higher in carbohydrates, added sugars, and fiber [6,13,15,18]. Another study demonstrated that most snacking patterns among adults were correlated with higher intakes of potassium, calcium, fiber, vitamin A, and magnesium [19]. Similar results have been observed among older adults (over 65 years old) where snacking frequency was positively correlated with daily intakes of vitamins A, C, and E, magnesium, copper, potassium, and beta-carotene [20]. In order to take into account the overall diet quality, some studies have used the U.S. Department of Agriculture's Healthy Eating Index (HEI). Data from NHANES 1999−2004 showed that snacking was modestly but significantly associated with higher HEI scores and a slightly more nutrient-dense diet [21]. When analyses are made by studying the snacking patterns, results reported that several snacking patterns were associated with better diet quality than those consuming no snacks [19]. The only snacking pattern significantly inversely correlated with diet quality was the soft drink pattern. In terms of food groups, results from a cross-sectional study reported that only the percentage of energy from nuts, fruits, and juices was significantly positively associated with diet quality [10]. Conversely, snacking energy coming from desserts, sweets, and sugar-sweetened beverages was significantly inversely correlated with diet quality. When comparing snack choices between genders, it was noted that women were more likely to choose healthier snack foods while men were more likely to choose unhealthy snack foods such as sweets and savories [22].

In China, the most common snacks were fruits, grains, and beverages over all age groups [8]. Interestingly, the increase in snacking observed over the two last decades seems to be explained by a high consumption of fruit as snacks. In the Mexican population, fruit was also the most common snack among adults [7]. Otherwise, salty snacks, sweet snacks, sugar-sweetened beverages, and milk were frequently consumed as snacks. Similar results were reported in an article studying a population from Brazil [6]. The most commonly consumed snacks were sweets and desserts, fruit, sugar-sweetened beverages, and also sweetened coffee or tea and a typical Brazilian snack (*salgados*, which is a high-calorie fried/baked dough). While fruit is also a common snack in Great Britain, the most common snacks are confectioneries such as chocolate and biscuits [17]. It was noted that the most popular snacks are those which can be easily transported. In Canada, data demonstrated that more than 41% of the calories eaten between meals were coming from the "other foods" category such as high-fat and/or high-salt snack foods or beverages [14]. In Spain, results from a survey revealed that snacking was positively associated with regular consumption of salty and fatty foods [23]. In the United States, total fruit, whole fruit, whole grains, milk, oils, and sodium component scores were positively associated with snacking frequency [21]. In the United Kingdom, an interaction between BMI and snacking frequency has been noticed [4]. Thus, differences in the types of snacks consumed were examined by BMI. Results showed that overweight individuals had higher intakes of crisps, chocolates, ice cream, and sweets and lower intakes of yoghurt and nuts when compared with normal-weight individuals.

Regarding the food forms, data from NHANES 2006 showed that food-only snacks increased by 180 kcal per day for both children and adults while adults only consumed on a daily basis approximately 100 kcal more from snack beverages when compared to 1977 [16]. Over the years, the percentage of snack calories coming from beverages remained essentially the same for all age groups. However, this percentage was lower in children (∼25% of all snack calories) than in adults (∼35% of all snack calories). As for the energy density coming from snack beverages, it has increased over the years in all adults [24]. Moreover, it has been noted that the number of snack episodes and the duration of each eating period increased with the daily energy intake from beverages [25]. Consequently, beverage-only snacking episodes tended to result in increased energy intake over the day.

Results from these studies suggest that snacking may be compatible with an adequate diet quality [9]. However, snacks are increasingly considered as an important part of eating patterns, which may involve either favorable (healthy and/or nutrient-dense) or less favorable (unhealthy, low nutrient, and/or high energy-dense) foods [15,22].

V SNACKING AND ENERGY BALANCE

Over the last decades, eating frequency and energy intake from snacks have both increased and obesity has been on an upward slope. On a theoretical basis, daily total energy intake varies according to eating events as well as energy intake and/or energy density during these eating occasions. That said, a high number of eating events may not

necessarily promote a positive energy balance if they are low in energy content. Nevertheless, it has been shown that caloric intake within an eating occasion is influenced by the portion size in both children and adults leading to overconsumption [1,26]. Therefore, even though it is widely assumed that larger portion sizes contribute to high rates of overweight and obesity [27], it is not clear whether or not caloric compensation (e.g., reduction of intake in main meals) occurs when snacks are consumed.

For both adults and children, studies reported that a greater eating frequency or a "snacking pattern" may promote a positive energy balance [13,19,28–31]. The number of snacks was directly related to the daily energy intake indicating that snack consumption is not entirely compensated by reduced food intake during main meals [28]. Among adolescents and adults, an increase in energy intake from snacks was a result of greater snacking frequency and also, larger snack portion size [32,33]. However, eating frequency was also negatively associated with energy intake per eating occasion [6,31] and greater eating frequency was reported to not correlate with a higher BMI in adults [22,31,34] and children [35].

The first part of this section [Beneficial Effect or No Effect of Snacking on Energy Balance (Weight Management) and Negative Effect of Snacking on Energy Balance (Overweight and Obesity)] presents more detailed studies that investigated the association between snacking and body weight or BMI. A more important focus was directed toward data published after 2010 within this section, but results from older studies can also be found in Tables 25.2 (children) and 25.3 (adults). The second part of this section (The Benefits and Risks of Snacking: Possible Explanations and Proposed Mechanisms) presents factors explaining why snacking may be perceived as both beneficial and deleterious for weight management.

A Beneficial Effect or No Effect of Snacking on Energy Balance (Weight Management)

Several studies reported a beneficial role of snacking on energy intake and thus on energy balance suggesting that snacking may be helpful in the context of body weight management. Most of these studies used a cross-sectional or prospective design but there are also a few intervention studies supporting this outcome.

Adult cross-sectional studies have reported either beneficial or neutral roles of snacking on energy balance. One of these studies verified dietary patterns and their associations with BMI [34]. The "snack pattern" was significantly correlated with a lower BMI. Another group of researchers compared normal-weight individuals to weight loss maintainers and overweight individuals [55]. It has been observed that self-reported eating frequency—including meals and snacks—was higher among normal-weight and weight loss maintainers than in overweight participants and there were no differences in the number of meals consumed. Authors further concluded that eating frequency may be an important aspect for weight loss maintenance. Differences between obese and nonobese individuals have also been noticed through another cross-sectional study showing that normal-weight subjects consuming additional snacks were associated with lower body fat [4]. Hence, these results suggest a beneficial impact of snacking on weight management both for obese and nonobese individuals.

Neutral effects of snacking on energy balance have also been reported. Data from a food panel questionnaire completed by more than 6000 participants evaluated the association between snack frequency (number of additional food items consumed between main meals) and BMI [22]. Results indicated that there was no association between snack frequency and BMI. Among women 40–60 years old, a study revealed that eating frequency which included meals and snacks was not associated with overweight and was not different between BMI groups [31]. Conversely, eating frequency was positively associated with total energy intake but negatively associated with energy intake per eating occasion regardless of BMI. Another cross-sectional study investigated dietary patterns at two different European universities and their relationship to BMI [58]. There were no general relationships between BMI and snacking regardless of the country. Thus, results from these cross-sectional studies in adults demonstrated that consumption of snacks is not associated with a higher BMI and might help individuals maintain their weight loss.

The beneficial or neutral impact of snacking on energy balance observed in adults has also been observed among children and adolescents. For example, Snoek and colleagues [37] noticed that high snacking frequency was associated with a lower risk of being obese compared to low snacking frequency in children or adolescents. Another cross-sectional study performed in 5811 American adolescents showed that snacking frequency and percent energy from snacks were associated with a lower prevalence of overweight/obesity regardless of reported efforts to lose weight in some adolescents [41]. Kelishadi and colleagues observed similar results in another cross-sectional study done in 14,880 children and adolescents [47]. In this study, snackers versus nonsnackers were less likely to be overweight/obese and to present abdominal obesity. This is in agreement with two other studies, which demonstrated that obese children were more likely to be nonsnackers compared to the normal-weight children [43] (done in 4262 Chinese children and adolescents) or to have no morning snacks [45]. Other results even showed that meal

TABLE 25.2 Association Between Snacking and Body Weight in Children/Adolescents

Study	Population	Design	Snacking Definition[a]	Summary of the Results	Impact on BW[b]
Crooks [134]	U.S. elementary school children ($n = 54$), grades 3–5	Cross-sectional	Type of food and its nutrient profile	High average daily snack consumption was reported in boys "at risk" for overweight, but not girls (probably because of the small number of participants)	+
			Measures: four 24-hour dietary recalls		
Kant [36]	American children and adolescents ($n = 4852$), aged 8–18 years	Cross-sectional	Type of food (i.e., low-nutrient-density foods) and its nutrient profile (e.g., visible fat; table sweeteners, candy, and sweetened beverages; baked and dairy desserts; salty snacks; and miscellaneous)	High amounts of low-nutrient-density food intake was related to higher energy intake and lower amounts of the five major food groups and most micronutrients but not body weight	≠
			Measures: 24-hour dietary recall		
Nicklas et al. [135]	Euro-American and African-American children ($n = 1562$), aged 10 years	Cross-sectional	Type of food and its nutrient profile (e.g., salty snacks, candy, desserts, fats/oils, and sweetened beverage groups)	Total amount of food consumed, notably from snacks, was positively associated with overweight status	+
			Measures: 24-hour dietary recall		
Huang et al. [136]	U.S. children ($n = 1995$), aged 3–19 years	Cross-sectional	Type of food and its nutrient profile	Percent energy from snacks was not associated with BMI in plausible reported energy intake from children	≠
			Measures: two 24-hour dietary recalls	Energy intake and meal patterns were associated with BMI only in plausible reported energy intake from children (compared to the total sample)	+
Snoek et al. [37]	Dutch adolescents (girls $n = 4581$; boys $n = 4430$), aged 11–16 years	Cross-sectional	Number of eating events (snacks)/day	Frequent snacking (three or more snacks per day) was associated with a lower risk of being overweight compared to less frequent snacking	−
			Measures: self-reported	No association between snacking and overweight was found in multivariate analyses, which included all eating and lifestyle behaviors in girls	≠
Lioret et al. [38]	French children ($n = 748$), aged 3–11 years	Cross-sectional	Type of food and its nutrient profile (e.g., French fries, soft drinks, sweetened beverages, low consumption of yogurt/cottage cheese)	The "snacking and sedentary pattern" was positively associated with childhood overweight in the youngest children	+
			Measures: parents for children >10-years old or self-reported 7-day dietary recall		

(*Continued*)

TABLE 25.2 (Continued)

Study	Population	Design	Snacking Definition[a]	Summary of the Results	Impact on BW[b]
Dubois et al. [39]	French Canadian children ($n = 1549$), aged 4–5 years	Cross-sectional	Eating frequency (snacks) and watching TV behavior Measures: maternal self-administration questionnaire	Snacking while watching TV on a daily basis was associated with higher average BMI than snacking less frequently in front of the TV	+
Macdiarmid et al. [40]	Scottish children ($n = 156$), aged 5–17 years	Cross-sectional	Eating frequency and food items (core food groups, e.g., pasta, bread, eggs, etc., vs noncore food groups, e.g., biscuit, pudding, yogurt, soft drink, etc.) Measures: 4-day unweighted dietary recalls	Meal and snack frequency was not different between BMI groups (normal weight vs overweight/obese)	≠
Kerr et al. [33]	Britain adolescents ($n = 434$) and Northern Ireland adolescents ($n = 47$), aged 13–16 years	Cross-sectional	Eating frequency outside three regular meals Measures: 7-day dietary recall	Snack energy intake, percent of energy from snack or frequency of snack was not different between normal weight and overweight/obese adolescents	≠
Keast et al. [41]	U.S. adolescents ($n = 5811$), aged 12–18 years	Cross-sectional	Number of eating occasions or the time of day food was consumed	Increased snacking frequency and increased percentage of energy from snacks was associated with a lower prevalence of overweight or obesity and abdominal obesity	−
		Data from NHANES	Measures: self-reported 24-hour food recall	Snackers, compared with nonsnackers, were less likely to be overweight or obese and less likely to have abdominal obesity	
Mushtaq et al. [42]	Pakistani primary school children ($n = 1860$), aged 5–12 years	Cross-sectional	Type of food consumed as snacks and its nutrient profile Measures: self-reported (interview)	Eating snacks and fast food ≥ once a week (43%) was more likely to be associated with overweight and obesity	+
Guo et al. [43]	Chinese children and adolescents ($n = 4262$), aged 5–18 years	Cross-sectional	Type of food consumed as snacks Measures: self-reported	Obese participants were more likely to be nonsnackers compared to the normal-weight ones	−
Jennings et al. [44]	UK children ($n = 2064$), aged 9–10 years	Cross-sectional	Time of day food is consumed, that is, foods or drinks consumed at any time other than meal periods Measures: self-reported 4-day dietary journal with parental assistance	Increased snacking frequency was favorably associated with adiposity, diet quality, and activity behaviors in healthy weight children but not centrally obese children	−

(*Continued*)

TABLE 25.2 (Continued)

Study	Population	Design	Snacking Definition[a]	Summary of the Results	Impact on BW[b]
Thibault et al. [45]	French children ($n = 4048$) aged 5–7 years and ($n = 3619$) aged 7–11 years	Cross-sectional	The time of day food is consumed Measures: self-reported or parents report morning snack yes/no	No morning snack was associated significantly and independently with higher overweight or obesity prevalence (as well as female gender, low or medium parental SES, never or sometimes having breakfast, never eating at the school canteen) in 7 to 11 years old	−
Murakami and Livingstone [46]	British children ($n = 818$) aged 4–10 years and adolescents ($n = 818$) aged 11–18 years	Cross-sectional	Eating frequency, that is, the number of eating events per day, except for those providing <210 kJ of energy Measures: self-reported and/or parent reported, 7-day diary	A higher eating frequency is associated with a higher BMI z-score in adolescents but a lower total cholesterol and LDL-cholesterol concentrations in children	+
Kelishadi et al. [47]	Iran children and adolescents ($n = 14,880$), aged 6–18 years	Cross-sectional	Eating frequency, that is, the number of eating events per day including snacks (e.g., cakes, chips, sugar-sweetened beverages, milk and dairy products, and sausages) Measures: parents report FFQ and self-reported FFQ for snack items	Higher eating frequency was associated with lower mean values of anthropometric measures (e.g. weight, BMI, WC)	−
Murakami and Livingstone [137]	British children ($n = 818$), aged 4–10 years and adolescents ($n = 818$), aged 11–18 years	Cross-sectional	Eating frequency and time of the day (meal = eating episode comprising ≥15% of TEI); snack = all other eating episodes (except food items <210 kJ of energy) Measures: self-reported and/or parent report, 7-day diary recall	Measures of meal frequency and snack frequency showed no association with adiposity measures in both children and adolescents; however, they were all associated with unfavorable dietary intake patterns Decreasing the number of small eating occasions regardless of the time of day may be important to improve diet quality but not adiposity	≠
Locard et al. [138]	French children obese ($n = 327$) and nonobese ($n = 704$), 5 years	Case–control	Not specified Measures: parents report	Snacking was identified as an environmental factor, which contributes to child obesity (along with southern European origin of the mother, excessive TV viewing and more importantly, short sleep duration)	+

(*Continued*)

TABLE 25.2 (Continued)

Study	Population	Design	Snacking Definition[a]	Summary of the Results	Impact on BW[b]
Tanasescu et al. [48]	Puerto Rican children ($n = 53$), aged 7–10 years	Case–control	Eating (snack) frequency (e.g., sweets and snacks: candy, chocolate, milk custard, jello, ice cream, sugared cookies, chips, peanuts, peanut butter, popcorn)	Snack consumption (i.e., sum of frequency of chips, peanuts, peanut butter, and popcorn consumption) was positively associated with BMI	+
	Obese ($n = 29$) and nonobese ($n = 24$)		Measures: self-reported FFQ and assisted 24-hour recall		
Field et al. [49]	Children (girls $n = 8203$ and boys $n = 6774$), 9–14 years of age	Prospective	Portion size and food items (e.g., one pop tart, one snack cake, three cookies, one small bag of popcorn)	Snack food intake was a significant, but weak, inverse predictor of annual changes in BMI z-score among the girls but not in boys (after controlling for Tanner stage of development, age, height change, activity, and inactivity)	−
			Sugar-sweetened beverages (e.g., soda) were not included as servings of snack foods	The authors conclude that although snack foods may have low nutritional value, they were not an important independent determinant of weight gain	
			Measures: self-reported FFQ (YAQ)		
Phillips et al. [50]	Adolescents (girls $n = 196$), aged 8–12 years	Prospective	Portion size and food items (e.g., baked goods, ice cream, chips, sugar-sweetened soda, candy)	Energy-dense snack food consumption was not associated with body weight status or fatness changes over the adolescent period	≠
			Measures: self-reported FFQ (Willett)	Soda consumption was the only snack food positively associated with body weight status changes (but not fatness)	
				Energy-dense snack food consumption was positively associated with TV viewing	
Thompson et al. [51]	American girls ($n = 101$) aged 9–15 years	Prospective	Time of the day and eating frequency	Eating frequency was significantly associated with change in BMI z-score during weekdays. Girls eating 4.0–5.9 times per day exhibited a smaller increase in BMI z-score than girls eating 6.0 or more times per day	+
			Measures: two 7-day dietary records	Eating frequency in the evening/night during weekdays also was significantly associated with change in BMI z-score	

(*Continued*)

TABLE 25.2 (Continued)

Study	Population	Design	Snacking Definition[a]	Summary of the Results	Impact on BW[b]
Li and Wang [52]	Low-income African-American adolescents ($n = 181$), aged 10–14 years	Prospective	Portion size and food items (e.g., one pop tart, one snack cake, three cookies, one small bag of popcorn)	High intake of snacks (as well as energy, fiber, fried food) was less likely associated with high BMI (same quartile at baseline and 1-year follow-up)	−
			Measures: self-reported FFQ (YAQ)	Decreases in snack intake (as well as energy) was negatively related to high BMI (same quartile at baseline and 1-year follow-up)	
Ritchie [53]	American girls ($n = 2372$) aged 9–10 to 19–20 years	Prospective	Eating frequency and time of the day (meal = eating episode comprising ≥15% of TEI); snack = all other eating episodes	Less frequent eating predicts a greater gain in adiposity in adolescent females	−
			Measures: self-reported 3-day diary recall each year except years 6 and 9		
Shroff et al. [54]	Colombian children ($n = 961$), aged 5–12 years	Prospective	Portion size and food items (e.g., candy, ice cream, packed fried snacks, soda, and sugar-sweetened fruit-flavored drinks)	Snacking patterns tend to be positively associated with BMI and adiposity changes	+
			Measures: self-reported FFQ	Soda intake was the only food pattern positively and significantly associated with BMI changes	

[a]*Snacking definition: (1) time of day food is consumed, (2) portion size + food items, (3) time and portion size and food items, (4) the number of eating events per day (or eating frequency), (5) consumer self-report, (6) type of food and its nutrient profile, (7) where eating occurs, and (8) physiological responses.*
[b]*Impact of body weight (BW): ≠, no association; +, positive association; −, negative association.*
BMI, body mass index; FFQ, food frequency questionnaire; YAQ, Youth Adolescent Questionnaire; TEI, total energy intake.

and snack frequencies were not different between normal-weight or overweight/obese children [40]. Although less numerous, some longitudinal studies showed that high snack food intake [49] or high eating frequency [53] were significant inverse predictors for BMI changes, although these were not strong relationships [49]. Increased snacking has been associated with lower BMI at baseline and 1-year follow-up in 181 low-income African-American adolescents [52]. A review of 32 cross-sectional and longitudinal studies investigating the association between snack consumption and energy balance concluded that most studies (21 out of 32 studies) found either a beneficial or a neutral impact of snacking on energy balance [13]. This is also concordant with a meta-analysis, which established that eating frequency was associated with lower body weight status in children and adolescent boys ($n = 3408$) and girls ($n = 3462$) [35]; however, this meta-analysis included some studies that considered only formal meals and not snacking in eating events.

Overall, these cross-sectional and longitudinal results support the notion that snacking in adults, children, and adolescents could be part of a healthy lifestyle with neutral or even beneficial effect on energy balance. However, given the nature of the studies, no causal relationships can be concluded. There are only few intervention studies, which evaluated the association between snacking and energy balance in adults. One of them verified the long-term (12 weeks) impact of three different snack foods, that is, nuts, chocolate, or chips (vs no snack food) on energy compensation and food habits among adult nonobese participants [64]. Results showed that energy compensation occurred for all types of snack food. Similarly, an intervention study also involving nonobese subjects evaluated the impact of an 8-week protocol of snack

TABLE 25.3 Association Between Snacking and Body Weight in Adults

Studies	Population	Design	Snacking Definition/ Measures[a]	Summary of the Results	Impact on BW[b]
Bachman et al. [55]	Weight loss maintainers ($n = 96$), normal weight ($n = 80$) and overweight ($n = 81$), aged ≥ 18 years	Cross-sectional	Consumers' self-report Measures: 24-hour dietary recalls	Eating frequency was higher in weight loss maintainers and normal-weight individuals than in overweight individuals	−
Barnes et al. [10]	Adults ($n = 233$), aged 18–60 years	Cross-sectional	Consumers' self-report Measures: three telephone-administered 24-hour dietary recalls	Snacking energy or frequency of snacking was not significantly associated with BMI	≠
Basdevant et al. [139]	Obese women ($n = 273$), aged 18–65 years	Cross-sectional	Time of day food was consumed Measures: dietary histories performed by trained dietitians	Snackers had higher total daily energy intakes compared to nonsnackers because of greater consumption of food at meals and between meals	≠
Cho et al. [34]	Adults ($n = 1118$), aged 30–70 years	Cross-sectional	Type of food and its nutrient profile, that is, three dietary patterns (e.g., vegetable-seafood, meat-fat, and snack) Measures: validated self-administrated food frequency questionnaire aimed at foods consumed during the previous year	Snack dietary patterns showed no association with obesity	≠
Drummond et al. [140]	Women ($n = 47$), aged 20–55 years	Cross-sectional	Time of day food was consumed	Women who ate most frequently also had the highest energy intakes, although this did not lead to higher BMI	≠
	Men ($n = 48$), aged 20–55 years		Measures: food diary, unweighted, recorded for seven consecutive days	In men, there was a significant negative correlation between eating frequency and body weight or BMI. As energy intake did not increase with eating frequency, men appeared to have compensated by reducing the mean energy consumed per eating episode	−
Edelstein et al. [141]	Adults ($n = 2034$), aged 50–89 years	Cross-sectional	Consumers' self-report Measures: short mailed questionnaire asked "How many meals and/or snacks do you eat in a usual day?"	Eating frequency was not associated with BMI	≠
Fabry et al. [142]	Men ($n = 379$), aged 60–64 years	Cross-sectional	Time of day food was consumed and consumers' self-report Measures: interview with a trained dietitian	Men who ate three meals a day (or less) were more susceptible to have an excessive weight than those who ate five meals (or more)	−

(*Continued*)

TABLE 25.3 (Continued)

Studies	Population	Design	Snacking Definition/ Measures[a]	Summary of the Results	Impact on BW[b]
Hampl et al. [143]	Men ($n = 1756$) and women ($n = 1511$), aged 18–65 years	Cross-sectional	Consumers' self-report and time of day food is consumed Measures: two nonconsecutive, multiple-pass 24-hour dietary recalls	Snacking patterns have some effects on energy and nutrient intakes but not on BMI	≠
Hartmann et al. [22]	Adults ($n = 6189$), aged 20–99 years	Cross-sectional	Consumers' self-report Measures: food frequency questionnaire	No association between snack frequency and BMI	≠
Howarth et al. [144]	Adults ($n = 2685$), aged 20–90 years	Cross-sectional	Consumers' self-report Measures: two 24-hour dietary recalls	Eating frequency may play a role in increasing overweight and obesity	+
Kant et al. [145]	Men ($n = 2580$) and women ($n = 4567$), aged 25–74 years	Cross-sectional	Number of eating events per day (or eating frequency) Measures: one 24-hour dietary recall administrated by a trained dietitian at baseline	Eating frequency and BMI were inversely correlated in both men and women	−
Kant and Graubard [56]	Adults ($n = 39,094$), aged 25–74 years	Cross-sectional	Number of eating events per day (or eating frequency) Measures: 24-hour recall administered by a trained dietary interviewer	Number of eating and snack episodes were significant independent predictors of higher energy intake in both men and women. There was an inverse association between the number of eating and snacking episodes and obesity but that was not significant after adjustment for low-energy reporting	≠
Ma et al. [146]	Adults ($n = 499$), aged 20–70 years	Cross-sectional	Number of eating events per day (or eating frequency) Measures: three 24-hour dietary recalls	Greater number of eating episodes each day was associated with a lower risk of obesity	−
McCarthy et al. [147]	Adults ($n = 1379$), aged 18–64 years	Cross-sectional	Type of food and its nutrient profile, that is, 28 food groups such as savory foods, cheeses, beverages, etc. Measures: 7-day food diary	Greater consumption of savory snacks was associated with an increased likelihood of being classified as obese	+
Metzner et al. [148]	Men ($n = 948$) and women ($n = 1080$), aged 35–69 years	Cross-sectional	Number of eating events per day (or eating frequency) Measures: 24-hour dietary recall	Frequency of eating was inversely related to the adiposity index for men and women separately	−
Mills et al. [31]	Overweight and obese women ($n = 1099$), aged 40–60 years	Cross-sectional	Consumers' self-report Measures: one mailed 1-day food record	Eating frequency was not associated with overweight/ obesity but related to energy intake	≠

(*Continued*)

TABLE 25.3 (Continued)

Studies	Population	Design	Snacking Definition/ Measures[a]	Summary of the Results	Impact on BW[b]
Murakami and Livingstone [57]	Men ($n = 678$) and women ($n = 809$), aged 19–64 years	Cross-sectional	Type of food and its nutrient profile (<15% of total energy intake) and time of the day food was consumed Measures: 7-day dietary record	Higher snack frequency was associated with higher adiposity measures	+
Neuhouser et al. [149]	Adults ($n = 982$)	Cross-sectional	Type of food and its nutrient profile, that is, high-fat snacks, reduced-fat snacks, or free-fat snacks Measures: food frequency questionnaire and snack-food questionnaire	There was no significant correlation between snack intake and BMI	≠
Ruidavets et al. [150]	Men ($n = 330$), aged 45–64 years	Cross-sectional	Number of eating events per day (or eating frequency) Measures: 3-day food record	Number of eating occasions was negatively associated with BMI	−
Scherwitz and Kesten [151]	Adults ($n = 5256$)	Cross-sectional	Type of food and its nutrient profile ("Task Snacking" includes such behaviors as eating while working, in front of the computer or TV, driving, reading, talking on the phone, or walking) Measures: 80-item questionnaire, designed to measure food, nutrition, and eating themes	The results show that eating style "Task Snacking" was predictive of BMI	+
Spanos and Hankey [58]	University students ($n = 160$), aged ≤24 years	Cross-sectional	Consumers' self-report Measures: food frequency questionnaire comprised 16 questions assessing meal and snacking habits	No relationships were found between BMI and snacking	≠
Summerbell et al. [59]	Adults ($n = 187$), aged 17–91 years	Cross-sectional	Time of day food is consumed Measures: 7-day weighed dietary records	Feeding pattern was not related to BMI within the working age or elderly groups. In the middle-aged group, lower energy intakes during the evening were associated with lower BMI	≠
Titan et al. [152]	Women ($n = 7776$), aged 45–75 years	Cross-sectional	Consumers' self-report	Increased eating frequency was weakly but significantly and positively associated with BMI	+
	Men ($n = 6890$), aged 45–75 years		Measures: food frequency questionnaire	Increased eating frequency was weakly but significantly and negatively associated with BMI	−

(*Continued*)

TABLE 25.3 (Continued)

Studies	Population	Design	Snacking Definition/ Measures[a]	Summary of the Results	Impact on BW[b]
Wahlqvist et al. [153]	Men ($n = 145$) and women ($n = 148$), aged 70 years and over	Cross-sectional	Number of eating events per day (or eating frequency) Measures: diet history	Consumption of a greater number of meals/snacks was negatively associated with the body fatness	−
Whybrow and Kirk [154]	Women ($n = 44$), aged 17–26 years	Cross-sectional	Number of eating events per day (or eating frequency) Measures: 7-day weighed food diary	There was a significant negative correlation between eating frequency and BMI	−
Zizza and Xu [21]	Adults ($n = 11,209$), aged 20 or older	Cross-sectional	Consumers' self-report Measures: 24-hour dietary recall	Snacking frequency was inversely associated with BMI	−
Berteus Forslund et al. [155]	Obese ($n = 83$) and lean ($n = 94$) women, aged 37–60 years	Case–control	Consumers' self-report Measures: meal pattern questionnaire	The number of reported intake occasions across a usual day was higher in obese women compared with controls and the timing was shifted to later in the day	+
Berteus Forslund et al. [156]	Obese men ($n = 1891$) and women ($n = 2368$), aged 30–60 years versus lean men ($n = 505$) and women ($n = 587$), aged 37–60 years	Case–control	Consumers' self-report Measures: self-administered meal pattern questionnaire	Obese subjects were more frequent snackers than lean subjects and women were more frequent snackers than men. Snacks were positively related to energy intake. Sweet, fatty food groups were associated with snacking and contributed considerably to energy intake	+
Bes-Rastrollo et al. [60]	University graduates ($n = 10162$)	Longitudinal prospective	Consumers' self-report Measures: semiquantitative food frequency questionnaire	Between-meal snacking was significantly associated with a higher risk of substantial weight gain. Usual snackers presented an adjusted 69% higher risk of becoming obese during follow-up	+
Keski-Rahkonen et al. [157]	Men ($n = 2060$) and women ($n = 233$), aged 16 years (T1), with follow-ups at 17 years (T2), 18.5 years (T3), and at 22–27 years (T4)	Longitudinal	Consumers' self-report Measures: eating style questionnaire at T4	Frequent snacks were significantly associated with obesity	+
Cameron et al. [61]	Obese men ($n = 8$) and women ($n = 8$), aged 18–55 years	Intervention	Number of eating events per day (or eating frequency) Measures: snacks included through a meal plan (three meals + three snacks per day vs three meals no snack)	Increasing meal frequency does not promote greater body weight loss during an 8-week energy-restricted weight loss program	≠

(*Continued*)

TABLE 25.3 (Continued)

Studies	Population	Design	Snacking Definition/ Measures[a]	Summary of the Results	Impact on BW[b]
Fay et al. [62]	Adults ($n = 50$)	Intervention	Type of food and its nutrient profile (e.g., M&Ms and Maltesers) Measures: snack provided by the research team	After eating until satiation, participants who initiated another snacking episode, their intake was predicted by higher BMI	+
Kong et al. [63]	Overweight or obese women ($n = 123$)	Intervention	Consumers' self-report Measures: food records and 24-hour recalls	Mid-morning snackers had a lower weight loss after a 12-month weight loss intervention when compared to nonmid-morning snackers	+
Pearson et al. [64]	Nonobese adults ($n = 102$), aged 18–65 years	Intervention	Type of food and its nutrient profile (e.g., hazelnuts, chocolate, potato crisps, or no added snack food) Measures: snacks provided by the research team for a 12-week period	Energy compensation occurred for all three snack conditions in this nonobese population	−
Piehowski et al. [65]	Overweight and obese women ($n = 26$), aged 25–45 years	Intervention	Type of food and its nutrient profile, that is, dark chocolate snack or a nonchocolate snack (e.g., nonchocolate sweet snack of fruit-flavored licorice) Measures: snacks provided by the research team for a 18-week period	Improvements in anthropometric and body composition measurements were achieved with a reduced-calorie diet including a snack	−
Viskaal-van Dongen et al. [66]	Nonobese men ($n = 16$) and women ($n = 66$), 18–35 years	Intervention	Time of day food was consumed + type of food and its nutrient profile, that is, snacks consumed with or between meals and snacks having a low (e.g., fruits or dairy products) or high (e.g., cereal products or savory snack) energy density Measures: snacks provided by the research team for a 8-week period	Consuming snacks did not contribute to weight gain. Healthy, nonobese young adults were able to maintain a normal body weight through an accurate compensation for the consumption of snacks	≠

[a] *Snacking definition: (1) time of day food was consumed, (2) portion size + food items, (3) time and portion size and food items, (4) the number of eating events per day (or eating frequency), (5) consumer self-report, (6) type of food and its nutrient profile, (7) where eating occurs, and (8) physiological responses.*
[b] *Impact of body weight (BW): ≠, no association; +, positive association; −, negative association.*

consumption on changes in body weight [66]. There were four parallel groups: snacks consumed with or between meals and snacks having a low or high energy density. No differences were noted in body weight between the four groups. In this study, moment of consumption and energy density did not influence body weight. Thus, this finding suggests that snacks (high or low in energy density) do not contribute to weight gain among nonobese young adults. In this situation, it is possible that nonobese adults were able to maintain a normal body weight through an accurate caloric compensation.

Among obese individuals, a study investigated whether having greater meal frequency (three meals + three snacks per day) could lead to a greater weight loss in

comparison with lower meal frequency (three meals per day) within an energy-restricted diet of 8 weeks [61]. In this case, increasing meal frequency did not compromise body weight loss compared with a no-snack pattern. Moreover, there were no significant differences between both groups for adiposity indices, appetite measurements, and gut peptides. Data collected from another intervention study among overweight adults aimed to verify the effects of a reduced-calorie diet including two different types of snacks, that is, chocolate or a nonchocolate snack on body composition [65]. This 18-week study showed a reduction in estimated daily energy intake for both groups. The intervention also led to a decrease in body weight, hip and waist circumferences, and fat mass with no change in lean mass. Authors then concluded that improvements in anthropometric and body composition can be achieved with a reduced-calorie diet even if snacks are included. These results suggest that snacking can be part of a weight loss program since energy restriction seems to be the primary independent determinant of weight loss in this context. Unfortunately, intervention studies investigating the impact of snacking restriction and/or mandatory snacking on total energy intake and body weight are clearly lacking in children and adolescents.

In summary, for both adults and children, there is evidence to support the notion that snacks may have a beneficial and/or neutral effect on energy balance. Studies presented in this section suggest that energy compensation occurs in response to snacking and that benefits of snacking can be observed in normal-weight individuals or in overweight and obese individuals on a calorie-restricted diet. Thus, conclusions suggest that it is possible to integrate snacks to a healthy lifestyle.

B Negative Effect of Snacking on Energy Balance (Overweight and Obesity)

Despite a certain number of studies showing a beneficial role of snacking on energy regulation and weight control, other data suggest that snacking contributes to weight gain and obesity [9,29]. It has been stated that regardless of the time of consumption or macronutrient composition, snacks exerted a weak satiety effect [29]. Thus, the energy intake related to snacks was not compensated for over the next meal and led to a positive energy balance compared with no-snack conditions. This is particularly true when snacking occurs in a nonhungry state or in nonusual snackers [67].

A cross-sectional study examined the associations between meal and snack frequency with dietary intakes and adiposity measures [57]. After adjustment for potential confounders, meal frequency, using a definition based on time, was positively associated with BMI and waist circumference (WC) in men only while there were no associations when a definition based on energy contribution was used. Snack frequency was correlated with both BMI and WC regardless of the definition of snacks in both men and women. Similarly, another cross-sectional study examining associations between snacking frequency and adiposity measurements observed an increased WC when overweight participants consumed higher quantities of snacks [4]. Similarly, a longitudinal study verified the association between snacking and weight gain and obesity in a middle-aged population [60]. At the beginning of the study, all participants were classified as normal-weight; however, after a mean follow-up of 4.6 years, 2.7% of the sample developed obesity (BMI over 30 kg/m^2). Usual snackers presented a 69% higher risk of becoming obese during follow-up. Thus, eating snacks has been considered as a potential risk factor for obesity. A cross-sectional study has found associations between unhealthy snacking and BMI [68] while another has shown that energy intake from snacks was not compensated for during main meals; however, the association with BMI has not yet been verified [28].

In children and adolescents, although more studies seem to demonstrate a beneficial or neutral effect of snacking on energy balance, some evidence also suggests that snacking promotes a positive energy balance. Accordingly, cross-sectional or case−control studies found that frequency of snacking [46], percentage of energy consumed as snacks or greater consumption of energy-dense snacks [42,48] were associated with overweight and obesity among some groups. Prospective studies have also shown that snacking frequency was associated with greater changes in BMI [51,54]. Among adults and children, although some cross-sectional and longitudinal studies have shown associations between snacking frequency and a greater BMI, the evidence remains inconclusive. Thus, larger trials and intervention studies must be done to confirm these effects.

Only a few intervention studies support the notion that snacking results in a positive energy balance and these have been done in adults. In this regard, an intervention study in overweight and obese women investigating the associations between snacking and weight loss during a 12-month weight-loss program found that the percentage of weight loss was significantly lower among mid-morning (10:30 to 11:29 a.m.) snackers compared to nonmid-morning snackers [63]. However, there were no significant differences in the percentage of weight loss when measured by snacking frequency. Authors concluded that future weight loss programs should consider not only snacking frequency, but also the timing and quality of snacks. In another intervention study, Fay et al. observed that among individuals who initiated snacking (after already having reached satiety), the intake of

calories was associated with a higher BMI [62]. This finding suggests that snacking initiation in the absence of hunger could be an important contributor to overconsumption.

To our knowledge, intervention studies supporting the notion that snacking results in a positive energy balance in children are not common, yet, some observation studies document the influence of environment. Accordingly, some environmental stimuli seem to influence the impact of snacking on diet quality and adiposity in children. For example, at least two studies have shown that an increase in snacking in front of the TV was associated with an increased risk of obesity in early childhood [39,69]. One of these studies, performed in 1549 children aged 4−5 years in Quebec, Canada, found that children who ate their snack in front of the TV once daily or more (17% of the sample, based on parental reports) had higher BMIs than those who ate while watching TV less than once a day; however, there was no association between hours of daily TV viewing and BMI [39]. Another study observed that snacking and sedentary patterns were positively associated with childhood overweight in the youngest when compared with the oldest [38] suggesting that sedentary behaviors could represent another stimuli influencing the associations between snacking and energy balance.

In summary, the literature presents evidence supporting that snacking can have both beneficial/neutral and negative impacts on energy balance. The evidence seems to be more controversial in adults than in children and adolescents. In younger children, more evidence seems to point in the direction of a beneficial or neutral effect of snacking, yet, to our knowledge no intervention studies have specifically addressed this issue in this population. Additional research is needed to identify possible explanations or mechanisms linking snacking with body weight.

C The Benefits and Risks of Snacking: Possible Explanations and Proposed Mechanisms

Many factors may explain why snacking can be viewed as both beneficial on body weight control and as a risk factor for weight gain and obesity. The first and probably the most obvious factor is related to variations in the definition and assessment of snacking used in studies. This issue has been discussed in the second section of this chapter (Section II: Definition of Snacking). In line with the definition issue, the diversity of variables used as an indicator of snacking pattern represents another factor. Studies reported outcomes such as the time of eating, percentage of energy consumed as snacks, nutrient composition of snacks, and the types of foods consumed as snacks (see, e.g., Tables 25.2 and 25.3). These variable descriptors may not be capturing the same eating patterns, and correlations among them are unknown. Others issues include concerns with "when" snacking occurs, "what" is eaten, "how" the snack is perceived, and "what" is done while snacking. These issues are described here.

1 Timing of Meals

The timing of snacking refers not only to when to consume the snacks between meals but also the overall distribution of snacking over the day. It has been proposed that providing snacks to offset variations in glucose and insulin, two well-known biochemical parameters affecting appetite control [29], may prolong the intervals between eating events and reduce energy intake at eating events. Although this hypothesis is interesting, it has not been validated in well-controlled experimental studies. Accordingly, Marmonier and colleagues [70] demonstrated that eating at different time points after a meal (i.e., 5 minutes before blood glucose peaked; 40 minutes after blood glucose peaked; or 2 hours before usual dinner time) had no impact on subsequent energy intake. Thus, in this study, snacking timed to match biochemical indices failed to decrease hunger, delay subsequent meal, and reduce dietary compensation for the snacks under all conditions.

Another aspect that has not been extensively studied related to the timing of snacking and energy balance in the timing of the snacking occasions. Hence, there is more and more evidence to identify the timing of food intake (i.e., when we eat, as a novel factor that promotes weight gain or resistance to weight loss). A study demonstrating that mice fed during the light phase (i.e., mice biological night phase) gained more weight on a high-fat diet than mice fed the same diet, but during the nocturnal phase, largely contributes to the demonstration of the effect of timing of food intake on body weight [71]. In humans, observational studies have shown that greater food intake in the evening was associated with an increased risk of overweight and obesity [72] and was positively associated with BMI, even after controlling for sleep duration and energy intake [73]. In line with this, a review highlighted that obese individuals are consistently characterized by a delayed pattern of eating [74]. More recently, two weight loss interventions with cross-over designs showed less body weight loss in the late eating condition compared to the early eating condition [75,76]. In another study, individuals who ate their largest meal after 3 p.m. lost less weight during a weight-loss trial than individuals who ate their largest meal before 3 p.m. [77].

Research on the Night Eating Syndrome (NES), an extreme example of delayed food intake, also supports the findings that late eating could be involved in obesity. NES is a disorder characterized by excessive evening and nighttime eating and is associated with insomnia, a

depressed mood and distress. Although, this problem is also observed in normal-weight individuals, the prevalence of NES is higher among overweight and obese individuals [78]. In line with this, results revealed that individuals characterized by night-eating behaviors (i.e., excessive evening eating and night-eating, but not presenting clinically with NES) have higher BMI and poorer metabolic health [79]. When examined prospectively, these individuals (normal-weight) tended to have a higher increase in BMI over 2 years [80]. Among night-eating symptoms, nocturnal ingestion and morning anorexia were the two symptoms that predicted an increase in percent change in BMI and WC, respectively. Other studies have shown that night-eating can lead to weight gain (4.3−4.5 kg over 3−6 years) [81−83].

Taken together, observational and experimental studies suggest time of snacking could influence the impact of snacks on energy balance. In line with this hypothesis, some observational studies indicated that snacking often occurs in the evening [6,8]. Only a few cross-sectional studies reported timing of snacking, yet, Thompson and colleagues presented some results which support the idea that late snacking is linked with body weight gain [51]. Accordingly, in their prospective study in 101 young girls, only the mean percentage of daily energy consumed in the evening/night was positively associated with change in BMI z-scores ($p = 0.039$) after controlling for baseline BMI.

To our knowledge there are very few intervention studies which have evaluated the impact timing of snacking per se on energy balance. In one study, eleven women were invited to change the timing of their snacking without changing total caloric intake or meal frequency for a 13-day period. More specifically they had to consume either a morning (10 a.m.) or evening (11 p.m.) snack in addition to their three regular meals, which they had to eat at their usual times [84]. Even though the conditions did not impact body weight or glucose metabolism, the evening snack condition decreased fat oxidation and increased total and low density lipoprotein (LDL) cholesterol. In another study, LeCheminant et al. used a randomized cross-over study to examine the effects of restriction of energy intake at evening/night (from 7 p.m. to 6 a.m.) among 27 healthy young males [85]. Each condition was maintained for a 2-week period. The results showed a significant decrease in energy intake (− 244 kcal) in the energy-restricted condition and a significant difference in weight change between the two conditions: a small body weight reduction (− 0.4 kg) was observed in the energy-restricted condition whereas weight gain was observed in the usual condition (+ 0.6 kg). Although these results provide some preliminary evidence that late snacking may contribute to weight gain or resistance to weight loss, other results do not support this hypothesis. In the intervention by [63], where weight loss was significantly less among mid-morning snackers compared to nonmid-morning snackers, eating snacks was related with a diet higher in fruits, vegetables and fiber-rich foods, which was also part of the dietary recommendations in this study.

It is important to note that these studies were not all well controlled for energy intake, physical activity, sleep, and other factors and had substantial methodological differences (e.g., energy restriction vs no energy restriction). Even though more research is needed to confirm the impact of timing of snacks on body weight, these results suggest that timing of snacking should be considered more when studying the impact of snacking on energy balance.

The mechanisms that drive the association between timing of food intake and energy balance are not well understood but changes in appetite control over the course of the day have been suggested as one factor. Accordingly, studies from De Castro et al. proposed that intake in the morning is associated with a lower total daily energy intake, while intake at night is associated with greater overall daily energy intake [86]. This phenomenon was explained by the fact that satiating properties of ingested nutrients decline over the course of the day, with foods eaten in the morning producing greater satiety as shown by a greater satiety ratio (i.e., duration of the after meal interval in minutes divided by the energy content of the meal) than foods eaten later in the day [86,87]. In addition to appetite control, other mechanisms involving sleep, metabolism, circadian rhythms, and genetic factors have also been proposed [77,88]. The possibility that snack quality of late-eaters may be a factor involved in these relationships may not be excluded. In line with this, results from the QUALITY cohort [89] showed that children with delayed meal-related distribution of energy intake also reported less healthy eating habits such as a greater percent of fat and sodium intakes, fewer servings of fruits and vegetables and a higher occurrence of unhealthy snacks (high sodium, high saturated fat, low fruits and vegetables, etc.) compared to children with more normal meal-related distribution of energy intake [90]. Snack quality will be further discussed in the next section.

2 Type of Snacks and Food Quality

The quality of snacks consumed may also explain the inconsistent results observed between snacking and body weight. Although snacking can improve diet quality by contributing to the intake of several vitamins and minerals such as vitamin A, potassium, magnesium [19,20] and increasing fruits, whole grains, and fibers [33,44], snacking has also been associated with less desirable dietary intake patterns such as higher total energy intake and higher added sugar in adults and children [15,57]. It is

also noteworthy that the type of snack has been associated with overall diet quality [10,33]. In terms of food groups, results from a cross-sectional study reported that only percentage of energy from nuts, fruits, and juices was positively associated with diet quality [10]. In contrast, snacking energy coming from desserts, sweets, and sugar-sweetened beverages was inversely correlated with diet quality. In the same study, general snacking behaviors (intake or frequency) were not associated with BMI. Percentage of snacking energy from vegetables was however significantly correlated with a lower BMI whereas percentage of snacking energy from desserts and sweets was positively associated with a higher BMI. In a study done in the United Kingdom, an interaction between BMI and snacking frequency was observed [4]. Thus, results revealed that overweight individuals had higher intakes of crisps, chocolates, ice cream, and sweets and lower intakes of yogurt and nuts when compared with normal-weight individuals. These foods generally have a higher energy density (kJ or kcal per gram), a food characteristic well known to influence energy intake. Foods that have a high energy density increase energy intake and vice versa [91]. This is concordant with the results of a review, which found that, in some groups of children, the percentage of energy consumed as snacks, or the consumption of energy-dense snack foods, was associated with a higher BMI [13].

Healthy snacking such as an increased intake of fruits, whole grains, and fiber could promote satiety and thus help body weight control. One example of that is the study of Waller et al. where 58 overweight/obese males and females with self-reported night snacking behaviors were randomized into a cereal group (CR) and no-cereal group (NC) for a 4-week period [92]. Compared to baseline, the CR group reduced their total daily caloric intake by almost 400 kcal whereas the NC experienced a small but nonsignificant reduction of energy intake during the same period. This study suggests that providing a structured healthy snack rich in fiber in the form of a "ready-to-eat" breakfast cereal can help to regulate energy intake and contribute to weight loss. Moreover, other studies showed that, compared with foods consumed at meals, snacks are actually higher in carbohydrates and lower in fat [15]. Higher eating frequency and replacement of fats by carbohydrates has been shown to be a successful weight management strategy [93].

It is also interesting to note that liquid snacks, more specifically consumption of sweetened beverages such as soda and juices, represent a type of snack that is more consistently associated with weight gain and obesity. According to one prospective study, energy-dense snack food consumption was not associated with body weight status or fatness over the adolescent period in girls [50]. However, soda consumption was the only snack food positively associated with body weight changes. More recently, Shroff et al. also observed that among all of the foods considered, soda was the only one that was positively associated with BMI changes in children whereas overall snacking patterns only tended to be associated with BMI and adiposity changes [54]. These results are concordant with a recent randomized trial among school-aged children which showed that sugar-sweetened beverages led to greater gains in BMI and WC compared with unsweetened beverages [94]. This suggests that liquid snacks could promote a greater risk of positive energy balance than solid snacks [95]. Although this hypothesis is still controversial, failure to compensate for energy from sugar-sweetened beverages at the subsequent meal compared to solid foods can be involved in this effect [96,97]. Moreover, it has been hypothesized that sugar-sweetened beverages bypass intake regulatory systems compared with solid foods and thus result in different physiological responses [96]. This will be discussed in the Section VI, Snacking as Part of a Healthy Diet.

Overall, these results suggest that high energy or low nutrient-dense snack foods promote body weight gain and obesity whereas low energy or high nutrient-dense snacks could help body weight control. Altogether, the evidence emphasizes the importance of considering the snack quality to identify the impact of snacking on energy balance.

3 Perceptions About Foods (Cognitive Bias/Cues)

Snacks foods are not equally viewed by everyone, and how a specific food is described can influence an individual's perception. A recent qualitative study reported that snack foods were not always perceived as "real food" by mothers [98]. In fact, when serving snacks, mothers more often attempt to manage their children's behavior rather than consider snacking as a way to provide nutritious foods. Different cognitive representations about what is a meal versus a snack may also influence intake. Capaldi et al. showed that when isocaloric preloads were given to participants, but presented either as a snack or as a meal, a lower intake was observed following the preload described as a meal than when described as a snack [99]. With regards to healthy eating, findings, from the literature that we recently reported in a review, suggest that various cognitive factors, such as type of food and branding, can significantly influence perceived healthiness of food via judgmental bias. However, impacts on food choice and intake are not always observed [100]. A study showed that perceiving a snack food (i.e., oatmeal-raisin cookies) as healthy significantly increased undergraduate female students' intake of that food by 35% (i.e., 56 kcal) during an ad libitum single meal in comparison to a condition in which the same food was perceived as

unhealthy [101]. The "healthy" snack was also seen as not only healthier but also more appropriate to eat. No differences between measured and self-reported snack intakes were observed which suggest that participants generally regarded it as normal to have a high intake of healthy foods. On the other hand, in a larger study conducted among men and women, verbal nutrition claims significantly influenced perceived healthiness of the snack food, but no impact was observed on food intake [102]. Wansink and Chandon have also addressed this issue, and reported an increased caloric intake of 28% among participants when chocolate candies were labeled as low in fat than when labeled as regular [103]. Overweight participants also appeared to be more inclined to eat in the low-fat labeling condition compared to the normal-weight participants, as their intake increased by 47% (vs 16% for normal-weight participants). A recent systematic review reported that attentional bias toward food cues varied between individuals, with an enhanced reactivity to food cues observed among overweight and obese individuals compared to normal-weight ones [104]. We are currently surrounded by numerous nutrition claims and logos that appear on food labels and advertising as well as by information published by the media or on various websites. All of these cognitive influences, often identified by the concept of "health halos," could positively influence energy balance while snacking. In fact, various food marketing strategies, such as branding, food claims, or food pricing, can bias food consumption and lead us to eat more than expected [105], and possibly without being aware of these overeating habits.

4 Attention Versus Mindless Eating

Recent work conducted by Susan Higgs suggests that when individuals' attention during eating is distracted by different factors, such as TV watching or computer games, their later snack intakes can be significantly increased [106]. On the other hand, when they are prompted to pay attention while eating, later snacking would decrease. This impact on snack intake can be explained by an impairment in the meal memory caused by the distractor, which is not observed when paying attention to eating [106]. In a recent systematic review and meta-analysis, evidence indeed support that being distracted while eating can have an impact on food intake, and positively influence energy balance [107]. While distraction induced moderate increases in immediate intake, the positive impact on later intake was greater. In another study, Robinson et al. examined if attentive eating could lead to reduced intakes among overweight and obese individuals [108]. In comparison to a control condition (neutral audio book), participants in the focused-attention condition, who ate their lunch meal while listening to audio instructions guiding them in eating attentively, ate 30% less when invited to snack 2–3 hours later. Behavioral strategies promoting greater attention while eating thus appear to be promising for appetite regulation. Altogether, these findings suggest that a snacking context that impedes the ability to pay attention while eating could be a potential risk factor for overconsumption. However, when eating attentively, snacking could be considered as a beneficial factor by enhancing reductions in energy intake.

5 Other Concerns

Besides different snacking definitions and assessments, timing, food quality, food perceptions, and level of attention while snacking, many other issues related to methodology and analysis could explain the discrepancy between studies investigating the impact of snacking on energy balance.

a Underreporting

Underreporting, particularly for foods rich in fat, sodium, or sugar, has been identified as one important issue in this regard. This occurs frequently in overweight and obese individuals and those involved in a weight loss program [109–111]. There is some evidence, which indicates that underreporting is especially common for snacks [112]. In a cross-sectional study conducted in American children and adolescents ($n = 4408$), different relationships were observed between snacking and BMI in plausible versus nonplausible reported energy intakes. For example, some relationships were found between the portion size of snacks (boys) or snack frequency (girls) and BMI percentile in the total sample whereas total snack consumption (frequency or size) was not significantly related to BMI percentile in the plausible sample. Similar results have been reported for eating frequency in studies examining adults as well [56]. When underreporters were excluded from studies, the originally reported inverse association between eating frequency and BMI was decreased or even reversed [8,59]. These findings suggest that excluding implausible energy intake reporters may be necessary when exploring the relationships between snacking and energy balance in both children and adults.

b Planned Versus Unplanned

The idea that the context of snacking "planned versus unplanned" may influence the effects of eating frequency and snacking on energy regulation has been proposed in a symposium on the subject in 2011 [30]. This theoretical model hypothesized that the inclusion of snacks may pose a moderate to high risk of overeating when a diet is not planned. Within a planned-diet, the risk of overeating remains low even when the snacking frequency is higher. The study of Waller et al. represents a good example [92].

In this study, overweight/obese males and females consumed either cereal as a snack or no cereal for 4 weeks. Total daily caloric intake was reduced by 400 kcal in the snack group while no significant reductions in energy intake was observed in the no cereal group suggesting that providing a structured snack in the "ready-to-eat" form would contribute to appetite control and body weight regulation.

This concept is also in line with the notion that compensation for energy intake is more often seen in habitual snackers (i.e., where a snack is usually planned in the daily eating pattern). In a 3-week intervention study where habitual/regular snackers were asked to consume several snacks up to 25% of their total energy intake, adequate energy compensation was observed [113]. This was more specific to low-fat snacks compared to high-fat snacks. In contrast, another experimental study showed that participants who never typically eat a snack in their daily routine and who were invited to eat a snack during the usual satiety period (i.e., when not hungry), snack intake did not lower hunger ratings, delay dinner request, and reduce energy intakes at dinner, leading to a higher total energy intake over the whole session [70]. Marmonier and colleagues showed that the absence of a satiety effect in individuals who never or rarely snacked could be biologically driven: usual snackers showing a decrease in hunger and lower glucose and insulin before snacking versus nonsnackers [70,114]. The evidence suggests that planned/regular eating habits may facilitate energy balance, while unplanned/irregular snacking, also characterized by eating in the absence of hunger, seems to be unfavorable to maintain energy balance [1]. Since the context of snacking, "planned versus unplanned," influences the impact of snacking on energy intake regulation, more studies should be done to assess this topic.

c Location

Another factor to consider is the location where the snack is consumed. Accordingly, the odds of eating an unhealthy snack appear to be 1.5 times greater when snacking occurs at places other than the home [68]. Furthermore, snacks taken from home were more often low-nutrient foods such as sugar-sweetened beverages. Indeed, snacks consumed during visits to private households and at restaurant outlets contained more energy, had a higher percentage of energy from fat and lower fiber density than snacks consumed at home [15]. Therefore, the place where a snack is consumed seems to influence the quality of the food eaten at these events.

d Physical Activity Level

Other factors identified which could explain mixed results around snacking and body weight regulation is related to the physical activity level (PAL) of snackers versus nonsnackers. This is supported by cross-sectional studies which have found either no relationship or a negative relationship between snacking and body weight and even if snacking was also related to higher energy intakes [36,41]. It is possible that snacking is associated with increased PAL, especially if sport supplements such as protein bars and sports drinks are considered as snacks [93]. The absence of a positive association between snacks and BMI could thus be explained by higher energy needs in more physically active individuals (lean or obese) that are met by a higher frequency of snacking. Besides PAL, this suggests that snacking is also associated with a more active lifestyle, which influences energy balance. Physical activity has not been considered in all snacking studies, and thus, results in this context should be interpreted with caution.

The results presented above emphasize the need to carefully interpret literature related to snacking and body weight regulation. Without knowledge about these potential factors, that is, underreporting, PAL, planned/regular snackers versus unplanned/irregular snackers combined with different population subgroups (e.g., lean vs obese), it is likely that associations between snacking and BMI will be variable.

VI SNACKING AS PART OF A HEALTHY DIET

Snacking represents one common eating pattern in many populations. Although some evidence suggests that spontaneous snacking increases the risk for weight gain, others indicate that snacking is associated with lower body weight and/or that it provides a substantial proportion of nutrients to the diets of both children and adults. In this context, it becomes important to identify how snacks can be integrated into a healthy diet. This will present some considerations related to each snack dimension in order to promote healthy snacking. Moreover, recommendations/strategies, which aimed to improve appetite control and to prevent overeating episodes, will be presented. These aspects could help health professionals in their interventions with their clients/patients to favor healthy snacking.

With respect to healthy eating, the composition of snacks is probably the most important aspect of snacking because this can have an impact on both appetite sensations and energy intake. Snack composition should be designed to increase satiation and satiety and help to regulate energy intake. Many food properties have been identified to influence appetite control. Accordingly, macronutrients have differing effects on satiety; protein being the most satiating and fat the least [115]. Preloads high in protein, when compared to meals lower in protein,

prolong satiety and reduce energy intake at a subsequent meal [116]. It seems that a minimum of 20 g of protein 30 minutes before a meal is necessary to represent the most effective strategy to enhance satiety [117]. Although there is evidence supporting the satiating effects of protein, studies that compare the satiety effects of snacks of various macronutrient compositions are limited. Marmonier et al. performed two experimental studies comparing the impact of isoenergetic high-protein, -carbohydrate, or -fat afternoon snacks on short-term appetite control [114,118]. They showed that although high-protein snacks prolonged the interval between preload ingestion and the subsequent meal (dinner), energy was not compensated, resulting in higher total energy intake. It must be noted that the snack items differed between conditions and thus different sensory characteristics or perceptions could have influenced the results. It is also unknown if the high protein satiating effect could have influenced long-term energy intake. Macronutrient composition is thus likely influenced by a snack's satiety properties, but additional studies are required to determine how to optimize these effects in the long term.

When considering carbohydrates, evidence has also shown that low glycemic index foods also improve appetite control [119], although this effect could also be due to other food properties such as energy density. Accordingly, as described earlier, high energy density increases the risk of overeating and favors a positive energy balance [120]. Thus, favoring low energy-dense snacks seems to represent one important strategy, which could help to achieve healthy eating. The mechanisms underlying the response to variations in energy density are not well understood but could involve sensory factors related to food volume, which influences gastric distension and gastric emptying rate, and/or cognitive factors, such as beliefs about the satiating capacity of different foods.

Decreasing the energy density of snacks can be easily achieved by including fruits or vegetables into a snacking episode. Besides their high content of water and fibers, fruits and vegetables are also high in nutrients. This is supported by studies, which showed that eating low energy-dense foods such as a salad or a soup before a meal (15−20 minutes) helps to reduce food intake during the meal [121,122]. Other long-term intervention studies showed that increasing fruits and vegetables as a nonrestrictive weight loss strategy over a 6-month period leads to a modest but significant weight loss [123]. This is concordant with a systematic review and meta-analysis revealing that increasing vegetable or fruit consumption tends to result in either a small reduction in body weight or reduced weight gain relative to the control group [124]. Yet, other studies indicate that advice to increase intake of fruits and vegetables can increase weight gain [125], weight regain [126], and more specifically in the overweight/obese population [127]. Increasing fruits and vegetables has been shown to be an effective strategy to decrease the consumption of unhealthy snacks among children [128]. Taken together, there is more evidence which support the recommendation of fruits and vegetables as a strategy to decrease energy density of snacks. However, it will be important to advise that this must be done in the context of energy balance where snacking is planned in the daily diet and this is probably more relevant in some populations (e.g., obese).

Formulating recommendations based on energy density to promote healthy snacking has some limitations. Accordingly, sweetened beverages such as soda are low energy-dense foods, yet it has also been associated with a positive energy balance and weight gain [96]. In contrast, nuts are high energy-dense foods due to their high fat and low water content. However, due to their strong satiety properties and the fact that their energy is not efficiently absorbed, evidence does not support their role in the risk of weight gain [129,130]. Moreover, advising the inclusion of low energy-dense foods as part of snacking without consideration for portion size can also promote a positive energy balance. It has been shown that portion size and energy density both influence energy (i.e., high energy density and larger portion size) and result in higher energy intakes [120]. Therefore, recommendations based on energy density should mostly consider foods that are relatively high in water and fiber as well as high in nutrients. Fresh fruits and vegetables, which usually come in reasonable portion sizes, meet these considerations.

The positive relationships between energy-containing beverage consumption and body weight and BMI in most epidemiological and intervention studies [96,131] highlight another important aspect related to the physical characteristics of the snack: solid versus liquid form. Although the exact mechanism is still being studied, solid seems to be more satiating than liquid especially among overweight individuals. This is also true when referring to fruits and vegetables. Accordingly, Houchins et al. compared the impact of energy matched liquid versus solid forms of fruits and vegetables in lean and obese individuals in randomized cross-over controlled trials [127,132]. They showed lower satiation when fruit beverages were consumed at a lunch visit compared with when solid fruits were consumed under comparable conditions, especially in the overweight/obese participants [132]. Moreover, the solid forms of fruits and vegetables improved dietary compensation and no weight change in the lean group whereas the overweight/obese group had lower compensation and significant weight gain. In contrast, liquid forms of fruits and vegetables induced incomplete dietary compensation and weight gain in both the lean and overweight/obese individuals [127]. Thus, evidence suggests that solid foods

instead of liquid foods should be recommended when snacking is planned. Again, increasing fresh fruits and vegetables consumption represents a strategy in line with this recommendation.

Besides energy density, studies related to timing of food intake suggest that limiting food intake or snacking later in the day (i.e., after dinner) could help to maintain or favor a negative energy balance. Of course this recommendation must be adapted according to cross-cultural differences (i.e., limiting "snacking" later in the day will not be the same in the United States vs Spain where the main eating event is around 6 p.m. in United States vs 10 p.m. in Spain). As explained in the previous section (Section V: Snacking and Energy Balance), timing of a given snack could influence its impact on energy balance. Timing is another factor to consider in favor of healthy snacking (Fig. 25.2).

Lastly, considering that perceptions and distractions can influence the amount of food eaten, it is important to include behavioral strategies that promote greater attention while eating and thus, appear to be promising for appetite regulation. These behavioral strategies are aimed to improve control around food selection and quantity [133]. Moreover, behavioral strategies should increase individual awareness about the impact of health or satiety claims on their behavior. Table 25.4 presents considerations and recommendations related to healthy snacking.

VII CONCLUSIONS

Given the important contribution of snacking to total energy intake and the need to develop more effective strategies to improve healthy eating habits and body weight management, it is important to better understand the impact of snacking on energy balance. Overall, the literature presents evidence that snacking is associated with both beneficial/neutral and negative effects on energy balance adults and children. Thus, the existing evidence does not clearly indicate that snacking is related to obesity and weight gain nor does it indicate that snacking is a good strategy for body weight control. Whether or not snacking influences energy balance requires considerations to numerous methodological issues such as snacking definitions and assessments, timing, food quality, food perceptions, and level of attention while snacking, underreporting, and other lifestyle habits. Future studies should consider the harmonization of the snacking definition as well as draw attention to better assess or control for these methodological issues. Because most of the studies have cross-sectional or prospective design, there is an urgent need to design intervention studies aimed at identifying the most optimal intervention strategies to promote healthy snacking patterns with a specific attention of snacking in children.

Although more studies are needed, the literature supports some strategies that can help to integrate snacking into a healthy diet without promoting positive energy balance. These strategies include the consumption of low energy and high nutrient-dense, solid snacks, such as fruits and vegetables, which are planned in the daily schedule and eaten in optimal environmental conditions. Even if they seem to be simple recommendations, significantly improving snacking patterns will likely require combined efforts of health professionals in the field of behavior modifications as well as those working in industry, schools, and other public health and community settings.

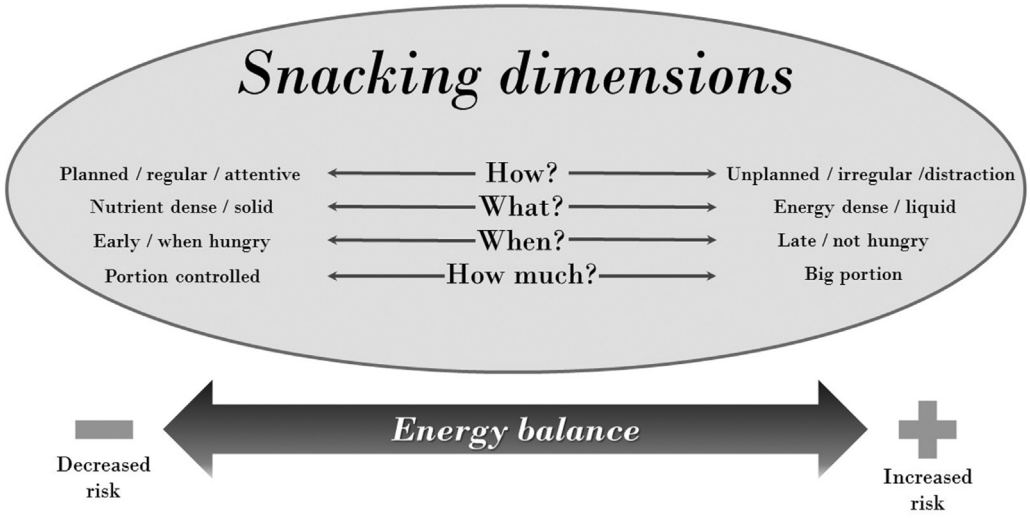

FIGURE 25.2 Summary-dimensions of snacking patterns.

TABLE 25.4 Considerations and Recommendations to Promote Healthy Snacking

Snacking Dimensions	Considerations	Recommendations
What?	What kind of food is usually eaten as snack? Is it usually low energy-dense and high nutrient-dense?	• Favor fruits and vegetables • Limit sweetened beverages as a snack • Other protein and nutrient-rich foods such as nuts
When?	When a snack is usually consumed?	• Limit snacking after dinner or in improper places • Do not store (tempting) foods in several places such as in the glove compartment of the car or the desk drawer at work. Keep these places snack-free • Do not snack if you are not hungry
How much?	Is a snack portion controlled? What is the number of snacks per day versus meals?	• Limit to one regular portion. Do not automatically use the suggested industry portion or whole package, but take your energy needs into account • Do not consume the total amount of a package or container of food but determine the amount of a "normal" serving size to eat • When there is a choice of portion size, pick the smallest one
How?	Is a snack planned in the diet? Is a snack consumed regularly? In a hungry state? How does snacking occur? Describe the context: In front of the TV? In the car? Is the person usually snacking? Is snacking part of an active lifestyle?	• Do not snack if it was not planned in your daily eating pattern unless the meal is delayed • Do not eat directly from the refrigerator or pantry • When preparing a snack, do not snack on the ingredients • Notice when you are satisfied and make it a habit to leave something on your plate when you are satisfied (satiated) • Avoid other activities such as watching TV, reading, or driving a car when eating • Avoid eating during work-related activities such as meetings, working at your desk, or making telephone calls • Take your time when eating your meal • Take time to appreciate the snack in a quiet environment

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Chapter 26

Genetic Influences on Blood Lipids and Cardiovascular Disease Risk

Toni I. Pollin[1], Jose M. Ordovas[2,3] and Martha Guevara-Cruz[4]

[1]University of Maryland School of Medicine, Baltimore, MD, United States, [2]Tufts University, Boston, MA, United States, [3]Centro Nacional Investigaciones Cardiovasculares (CNIC), Madrid, Spain, [4]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico City, Mexico

I INTRODUCTION

The major public health concerns in the developed world (i.e., cardiovascular disease (CVD), cancer, and diabetes) have both genetic and environmental causes. The interface between public health and genetics involves working toward an understanding of how genes and the environment act together to cause these diseases and how the environment (e.g., diet), rather than genes, might be manipulated to help prevent or delay the onset of disease.

CVD, the leading cause of mortality in most industrialized countries, is a multifactorial disease that is associated both with nonmodifiable risk factors, such as age, gender, and genetic background, and with modifiable risk factors, including elevated total and low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels, as well as reduced high-density lipoprotein cholesterol (HDL-C) level. Heritability estimates for blood lipids are high—approximately 40−60% for HDL-C, 40−50% for LDL-C, and 35−48% for TG [1].

Traditionally, the major emphasis of public health measures has been on lowering serum cholesterol, which has long been recognized as an important risk factor for the development and progression of atherosclerotic vascular disease; multiple clinical studies for the past 30 years have demonstrated the benefits of this approach. In view of these findings, the National Heart, Lung, and Blood Institute convened the National Cholesterol Education Program Adult Treatment Panel I (NCEP ATP I) [2]. This panel and similar panels throughout the world have set the standards for lowering lipid profiles in clinical practice. Subsequent revisions of these standards (NCEP ATP II and III) [3,4] have placed a greater focus on LDL-C, and target LDL-C levels are based on patient risk of subsequent coronary disease events. Since the publication of the NCEP ATP III guidelines, several large-scale clinical trials of cholesterol-lowering treatments have been conducted, the findings of which may be used to further refine current clinical practice standards [5,6]. Although most of the evidence that lowering serum LDL-C can reduce CVD morbidity and mortality comes from pharmacological interventions, the NCEP has emphasized that therapeutic lifestyle change should be the primary treatment for lowering cholesterol, and drug therapies should be reserved for cases in which lifestyle modification is ineffective. The lifestyle modifications advocated include dietary changes, increased physical activity, and weight management. The recommended dietary changes to reduce LDL-C include restriction of saturated fat to less than 7% of total caloric intake, restriction of cholesterol to less than 200 mg/day, increased viscous fiber intake (10−24 g/day), and increased plant stanol/sterol intake (2 g/day) [4]. However, it is not known how many individuals can achieve the recommended levels of serum lipids using this approach, in large part because it is currently impossible to predict plasma lipid response to dietary changes in individual patients [7,8].

Other pharmacological approaches to CVD risk reduction have been investigated, including treatments to lower serum TG and raise HDL-C. Fibrate drugs are most commonly used to lower TG. Fenofibrate therapy has been associated with changes of −25 to −59% in TG, −33 to +1% in LDL-C, and +1 to +34% in HDL-C in CVD

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patients [9]. The largest reduction of CVD risk by fenofibrate has been detected in patients with marked dyslipidemia, a feature of metabolic syndrome (MetS), in whom a 27% relative risk reduction was found in the Fenofibrate Intervention and Event Lowering in Diabetes study [10,11]; therefore, fenofibrate would be expected to have large beneficial effects in MetS patients. The use of fenofibrate in the management of lipoprotein disorders dates back to the mid-1960s. However, their prominence has lessened throughout the years because of unimpressive results in major clinical trials, safety concerns, and the emergence of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins). Moreover, the general trial results with these agents have been confusing, with varying cardiovascular benefits [12–14] depending on the dyslipidemia status [15].

The epidemiologic, experimental, and circumstantial evidence implicating low HDL-C as a major risk factor for CVD has driven considerable research on this lipoprotein fraction; it is hoped that raising HDL-C may further reduce CVD risk beyond what can be achieved with the use of statins. However, doubts about the clinical benefits of treatments to enhance plasma HDL-C levels have been raised by the premature termination of a large phase III trial with torcetrapib, the most potent and furthest-developed HDL-C-raising compound, which was discontinued because of excess mortality in patients receiving the drug [16]. The causes of torcetrapib failure are unknown and may be related to the drug's mode of action, to toxic off-target effects of the drug, or both. However, the failure of torcetrapib does not mean that targeting HDL in CVD prevention is no longer a research goal. Other HDL-C-raising therapies that act through similar or disparate molecular mechanisms are in various stages of preclinical and clinical development [17,18].

The considerable interindividual variation in lipid response, cardiovascular event response, and adverse events observed following these therapies has brought considerable attention to the concept of more targeted therapies based on genetic information. Pharmacogenomics (or pharmacogenetics) involves the search for and identification of genetic variants that influence patient response to drug therapy. During approximately the past decade, some progress has been made in our understanding of the variable efficacy of statin therapy [19]. Similarly, there is evidence that fenofibrate treatment improves the lipid profile, but there is significant interindividual variability in lipid response to fenofibrate, which may be mediated by specific genes [20,21]. Important limitations and issues in pharmacogenetics have been raised, however, which need to be resolved before its clinical application.

Regarding the connection between diet and plasma cholesterol concentration, several studies during the first half of the 20th century demonstrated that serum cholesterol could be modified by the composition of dietary fat [22,23]. Studies by Keys and colleagues [24] and Hegsted et al. [25] provided the first quantitative estimates of the relative effects of the various classes of dietary fatty acids and the amount of dietary cholesterol on serum cholesterol changes. Later, other predictive algorithms were developed, including predictions of LDL-C and HDL-C responses [26–28]. The relationships between dietary changes and serum lipid changes are well founded and predictable for groups. However, a striking variability in the response of serum cholesterol to diet between subjects was reported as early as 1933 [29], and this variability has been the subject of multiple reports. In some individuals, plasma total lipid and LDL-C levels dramatically decrease following the consumption of lipid-lowering diets, whereas they remain unchanged in others [27,30–34]. Multiple studies in animal models and in humans have shown that serum lipoprotein response to dietary manipulation has a significant genetic component [35–40]. Genetic variability could therefore have a significant impact on the success of public health policies and individual therapeutic interventions. Moreover, it could be partially responsible for the apparent lack of hard endpoint benefits shown by many dietary studies aimed at decreasing CVD [41–43].

As indicated previously, the success of CVD risk-reducing strategies has traditionally been measured based on their effects on plasma lipids and, specifically, lipoprotein levels. Lipoproteins are macromolecular complexes of lipids and proteins that originate mainly from the liver and intestine and are involved in the transport and redistribution of lipids in the body. Lipid and lipoprotein metabolism can be viewed as complex biological pathways containing multiple steps. Lipid homeostasis is achieved by the coordinated action of hundreds of gene products, including nuclear factors, binding proteins, apolipoproteins, enzymes, and receptors. Lipid metabolism is also closely linked to energy metabolism and is subject to many hormonal controls that are essential for adjustment to environmental and internal conditions. Genetic variability within candidate genes involved in lipoprotein metabolism has been associated with abnormal lipid metabolism and plasma lipoprotein profiles that may contribute to the pathogenesis of atherosclerosis. This complex regulatory network can be dissected into three major pathways (Fig. 26.1). The exogenous lipoprotein pathway describes the metabolism of lipoproteins synthesized in the intestine following dietary fat intake, the endogenous pathway involves the metabolism of lipoproteins involved in the transport of liver lipids to peripheral tissues, and the reverse cholesterol transport pathway describes the process by which excess peripheral lipids (primarily cholesterol) are transported to the liver for catabolism. Our knowledge about how variants in candidate genes

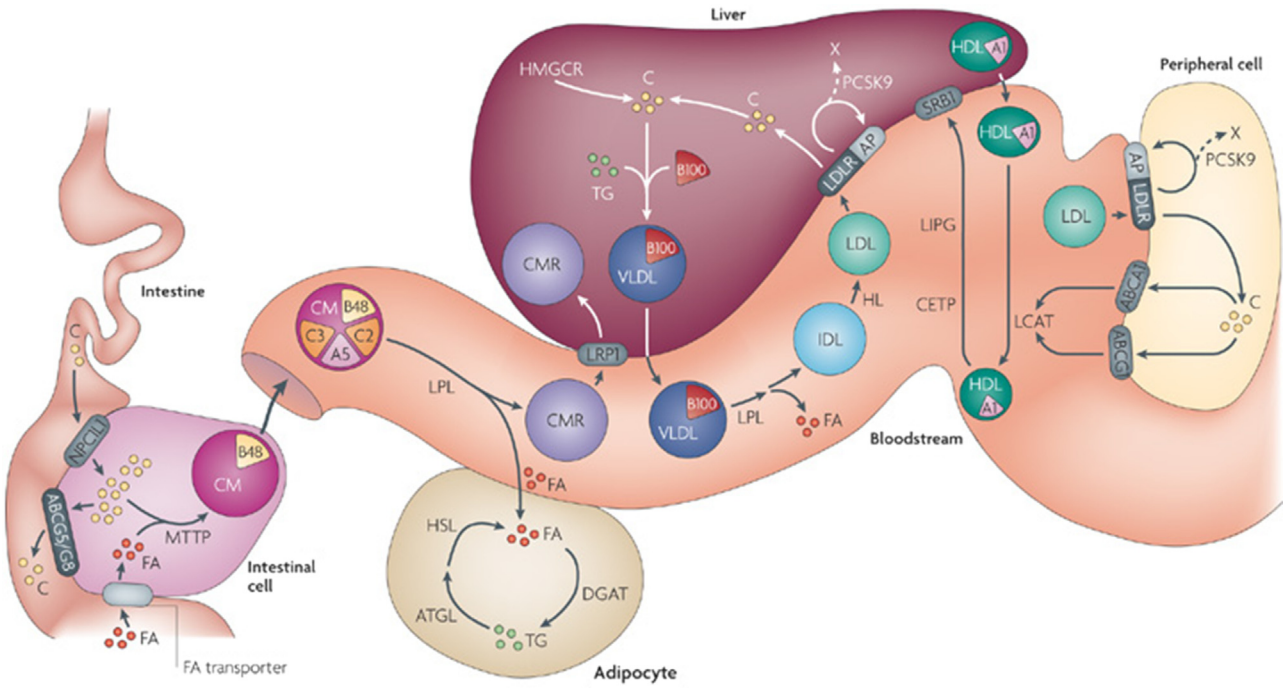

FIGURE 26.1 The main lipids in lipoproteins are free and esterified cholesterol (C) and TG. The metabolism of TG, LDL cholesterol, and HDL cholesterol is shown. In TG metabolism, hydrolyzed dietary fats enter intestinal cells (enterocytes) via fatty acid (FA) transporters. Reconstituted TG is packaged with C ester and the apolipoprotein B (APOB) isoform B48 into chylomicrons (CMs) by microsomal TG-transfer protein (MTTP) through a vesicular pathway. CMs, secreted via the lymphatic system, enter the vena cava and circulate until they interact with LPL, the secretion of which depends on lipase-maturation factor 1 (not shown), and which is secured to endothelium by proteoglycans and glycosylphosphatidylinositol-anchored HDL-binding protein 1 (not shown). CMs contain apolipoproteins, including APOA5 (A5), APOC2 (C2), and APOC3 (C3). Released free FAs incompletely enter peripheral cells. In adipocytes, enzymes including acyl CoA:diacylglycerol acyltransferase (DGAT) resynthesize TG, which is hydrolyzed by adipose TG lipase (ATGL) and hormone sensitive lipase (HSL). CM remnants (CMRs) are taken up by hepatic LDL receptor (LDLR), in the absence of LDLR they are taken up by LDLR-related protein-1 (LRP1). In liver cells (hepatocytes), TG is packaged with cholesterol and the APOB isoform B100 into VLDL; the TG contained in VLDL is hydrolyzed by LPL, releasing FAs and VLDL remnants (IDL) that are hydrolyzed by hepatic lipase (HL), thereby yielding LDL. In LDL cholesterol metabolism, sterols in the intestinal lumen enter enterocytes via the Niemann-Pick C1-like 1 (NPC1L1) transporter and some are resecreted by heterodimeric ATP-binding cassette transporter G5/G8 (ABCG5/G8). In enterocytes, cholesterol is packaged with TG into CM. In hepatocytes, cholesterol is recycled or synthesized de novo, with 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) being rate-limiting. LDL transports cholesterol from the liver to the periphery. LDL is endocytosed by peripheral cells and hepatocytes by LDLR, assisted by an adaptor protein (AP). Proprotein convertase subtilisin/kexin type 9 (PCSK9), when complexed to LDLR, short-circuits recycling of LDLR from the endosome, leading to its degradation (X). In HDL cholesterol metabolism, HDL, via APOA-I (A1), mediates reverse cholesterol transport by interacting with ATP-binding cassette A1 (ABCA1) and ABCG1 transporters on nonhepatic cells. LCAT esterifies cholesterol so it can be used in HDL cholesterol, which, after remodeling by CETP and by endothelial lipase (LIPG), enters hepatocytes via scavenger receptor class B type I (SRB1). *Reprinted by permission from Macmillan Publishers Ltd. R.A. Hegele, Plasma lipoproteins: genetic influences and clinical implications, Nat. Rev. Genet. 10 (2009) 109–121, copyright 2009.*

involved in each of these three interrelated processes affect dietary response and cardiovascular risk is discussed next.

A Exogenous Lipoprotein Pathway

The exogenous lipoprotein pathway begins in the enterocyte with the synthesis of chylomicron particles. Dietary fats absorbed in the intestine are packaged into large, TG-rich chylomicrons for delivery to sites of lipid metabolism or storage. During their transit to the liver, these particles interact with lipoprotein lipase (LPL) and undergo partial lipolysis to form chylomicron remnants. These chylomicron remnants pick up apolipoprotein E (APOE) and a cholesteryl ester from HDL, after which they are taken up by the liver in a process that is mediated by the interaction of APOE with hepatic receptors. In most people, this occurs quickly, and chylomicrons are not usually present in the blood after prolonged fasting. However, there is dramatic variability in postprandial lipoprotein metabolism, which is determined in part by genetic factors, and this variability could be highly relevant to achieving a more precise definition of individualized CVD risk [44,45]. The most relevant candidate genes involved in

this metabolic pathway and their known associations with plasma lipid levels and dietary response are described later.

B Endogenous Lipoprotein Metabolism

Hepatocytes synthesize and secrete TG-rich very low-density lipoprotein (VLDL), which may be converted first to intermediate-density lipoproteins and then to LDL through lipolysis by a mechanism involving LPL, similar to that described for the exogenous lipoprotein pathway. The excess surface components are usually transferred to HDL. Some of these remnants may be taken up by the liver, whereas others are further lipolyzed to become LDL, which, in humans, contains APOB as its primary apolipoprotein. This pathway uses many of the same genes as the exogenous pathway. The LDL receptor is primarily responsible for the catabolism of LDL particles in the liver and peripheral tissues. Another important factor in endogenous lipoprotein metabolism is the HMG-CoA reductase gene, which encodes a key enzyme in de novo cholesterol synthesis.

C Reverse Cholesterol Transport

Both the liver and the intestine synthesize HDL. In its precursor form, HDL is disc-shaped, and it matures in the circulation as it picks up unesterified cholesterol from cell membranes (see the later discussion of the ATP-binding cassette-1), other lipids (phospholipids and TG), and proteins from triglyceride-rich lipoproteins (TRL) as these particles undergo lipolysis. The cholesterol is esterified by lecithin−cholesterol acyltransferase (LCAT), and the small HDL3 particle becomes a larger HDL2 particle. The esterified cholesterol is either delivered to the liver or transferred by the action of the cholesterol ester transfer protein (CETP) to TRL in exchange for TG. The liver may then take up cholesterol via receptors specific for these lipoproteins, or it can be delivered to the peripheral tissues. The TG received by HDL2 is hydrolyzed by hepatic lipase, and the particle is converted back to HDL3, completing the HDL3 cycle in plasma. In the liver, cholesterol can be excreted directly into bile, converted to bile acids, or reused for lipoprotein production.

However, CVD has a multifactorial etiology in which a complex combination of environmental, genetic, and clinical risk factors seem to play determinant roles [46,47]. The complex architecture of this disease and its genetic basis [48] are still poorly understood, and some discrepancies remain in estimates of the heritability of numerous CVD-related quantitative traits (QTs) [49]. The identification and validation of genetic variants that promote CVDs and related QTs may lead to a better understanding of the pathogenesis of these diseases and to the identification of novel disease-associated biological pathways and risk factors.

During approximately the past two decades, the genetics of chronic diseases and related QTs have been assessed by candidate gene approaches [50] as well as linkage approaches, which were successful in families with Mendelian (single gene) forms of dyslipidemia, but less so in examining polygenic sources of lipid variation in the population. Twin and family studies have been used to estimate heritability, whereas population-based studies are used to identify variants that are associated with specific phenotypes and to explore interactions between genetic and environmental factors. Candidate genes are those considered to influence complex phenotypes due to their participation in related biological pathways or due to their location near a region of interest [50]. This hypothesis-driven research has provided the foundation for the development of genetic CVD risk profiles [51]. However, since 2007, genome-wide association studies (GWAS) have revolutionized the research of chronic diseases such as CVDs and their associated QTs [52−54]. Recent technological advances have enabled the rapid and accurate assessment of millions of single-nucleotide polymorphisms (SNPs) in large populations, and many new GWAS have been conducted for different CVD phenotypes and related traits. These studies have identified hundreds of largely common genetic factors, some of which have been frequently replicated, albeit with small individual effects. Moreover, GWAS have revealed previously unsuspected pathological pathways [55,56]. Nevertheless, the causal variants identified by GWAS are insufficient to explain the high heritability of different CVD phenotypes. Additional studies are required to further refine the prevention, diagnosis, treatment, and prognosis of CVD patients based on genetic factors [57]. For example, the decreasing costs of DNA sequencing technologies are enabling large-scale sequencing studies, including the entire genome or exome (region of the genome containing all protein coding genes), to emerge and identify rare and low frequency variants generally not identifiable via GWAS and likely to have larger individual effects.

II REPRESENTATIVE GWAS

Lipid measures represent excellent secondary phenotypes for the genetic investigation by many GWAS of CVD, and relatively large sample sizes can be obtained to study these traits. Here, we discuss the main features of several GWAS using lipid measures.

Sabatti et al. [58] reported GWAS results for nine quantitative metabolic traits (HDL-C, LDL-C, TG, glucose, insulin, C-reactive protein, body mass index, and systolic and diastolic blood pressure) in the Northern

Finland Birth Cohort of 1966 (NFBC1966), which was drawn from the most genetically isolated regions of Finland. This study replicated most previously reported gene associations and identified nine new associations, several of which highlighted genes with metabolic functions: HDL-C with *NR1H3* (LXRA), LDL-C with *AR* and *FADS1−FADS2*, glucose with *MTNR1B*, and insulin with *PANK1*. Two of these new associations emerged after adjusting results for body mass index. Gene−environment interaction analyses suggested additional associations that will require validation in larger samples. These loci, even when combined with quantified environmental exposures, explain little of the trait variation in NFBC1966. However, the association between LDL-C and an infrequent variant in *AR* suggests that cohort studies have the potential to identify associations with both common, low-impact and rare, high-impact QT loci.

In another study, Aulchenko et al. [59] reported the first GWA analysis of loci affecting total cholesterol (TC), LDL-C, HDL-C, and TGs sampled randomly from 16 population-based cohorts and genotyped using mainly the Illumina HumanHap300-Duo platform. Their study included a total of 17,797−22,562 persons aged 18−104 years and from geographic regions from the Nordic countries to southern Europe. They established 22 loci associated with serum lipid levels at a genome-wide significance level, including 16 loci that had been identified by previous GWAS. The six loci newly identified in this cohort were *ABCG5*, *TMEM57*, the *CTCF−PRMT8* region, *DNAH1*, *FADS3−FADS2*, and the *MADD−FOLH1* region. The effect sizes differed significantly by sex for three loci. Genetic risk scores based on lipid loci explained up to 4.8% of the variation in lipid profile and were associated with increased intima media thickness and coronary heart disease incidence.

To dissect the polygenic basis of these traits, Kathiresan et al. [60] conducted GWAS in 19,840 individuals and replicated it in up to 20,623 individuals. They identified 30 distinct loci associated with lipoprotein concentrations, including 11 loci that reached genome-wide significance. The 11 newly defined loci include common variants associated with LDL cholesterol near *ABCG8*, *MAFB*, *HNF1A*, and *TIMD4*; with HDL cholesterol near *ANGPTL4*, *FADS1−FADS2−FADS3*, *HNF4A*, *LCAT*, *PLTP*, and *TTC39B*; and with TGs near *AMAC1L2*, *FADS1−FADS2−FADS3*, and *PLTP*. The proportion of individuals exceeding clinical cutoff points for high LDL cholesterol, low HDL cholesterol, and high TGs varied according to an allelic dosage score. These results suggest that the cumulative effect of multiple common variants contributes to polygenic dyslipidemia.

Ma et al. [61] reported single-locus and epistatic SNP effects on TC and HDL-C using Framingham Heart Study (FHS) data. Single-locus effects and pairwise epistasis effects of 432,096 SNP markers were tested for their significance on log-transformed TC and HDL-C levels. Twenty-nine additive SNP effects reached single-locus genome-wide significance ($p < 7.2 \times 10^{-8}$), and no dominant effect reached genome-wide significance. Two new gene regions were detected: the *RAB3GAP1−R3HDM1−LCT−MCM6* region of chr02 for TC was identified by six new SNPs, and the *OSBPL8−ZDHHC17* region (chr12) for HDL-C was identified by one new SNP. The remaining 22 single-locus SNPs identified confirmed previously reported genes or gene regions. For TC, three SNPs identified two gene regions that were tightly linked with previously reported TC-associated genes, including rs599839 10 bases downstream of *PSRC1* and 3.498 kb downstream of *CELSR2*, rs4970834 in *CELSR2*, and rs4245791 in *ABCG8*. The association of HDL-C with *LPL* was confirmed by 12 SNPs 8−45 kb downstream, with *CETP* by two SNPs 0.5−11 kb upstream, and with the *LIPG−ACAA2* region by five SNPs in this region. Two epistasis effects on TC and 13 epistasis effects on HDL-C reached the significance of "suggestive linkage." The most significant epistasis effect ($p = 5.72 \times 10^{-13}$) was a dominant × dominant effect of HDL-C between *LMBRD1* (chr06) and the *LRIG3* region (chr12) and was close to reaching "significant linkage"; this pair of gene regions had six other D × D effects with "suggestive linkage." Genome-wide association analysis of the FHS data detected two novel gene regions with genome-wide significance, detected epistatic SNP effects on TC and HDL-C with the significance of suggestive linkage in seven pairs of gene regions, and confirmed the association of some previously reported gene regions with TC and HDL-C.

Teslovich et al. [62] identified additional common variants associated with plasma TC, LDL-C, HDL-C, and TG concentrations. They performed a meta-analysis of 46 lipid GWAS. These studies together comprised more than 100,000 individuals of European descent from the United States, Europe, and Australia. They reported 95 significantly associated loci ($p < 5 \times 10^{-8}$), with 59 showing genome-wide significant association with lipid traits for the first time. The newly reported associations include SNPs near known lipid regulators (e.g., *CYP7A1*, *NPC1LA*, and *SCARB1*) and in many loci not previously implicated in lipoprotein metabolism. The 95 loci contribute not only to normal variation in lipid traits but also to extreme lipid phenotypes, and they affect lipid traits in three non-European populations (East Asians, South Asians, and African Americans). That study also identified several novel loci associated with plasma lipids that are also associated with CVD. Finally, it validated the functions of three novel genes—*GALNT2*, *PPP1R3B*, and *TTC39B*—with experiments in mouse models. Taken

together, Teslovich et al.'s findings provided the foundation for a broader biological understanding of lipoprotein metabolism and identify new therapeutic opportunities for the prevention of coronary artery disease (CAD).

Lettre et al. [63] identified common genetic polymorphisms associated with CVD and its risk factors (LDC and HDL-C, hypertension, smoking, and type 2 diabetes) in individuals of African ancestry through a GWAS in 8090 African Americans from five population-based cohorts. They confirmed 17 loci that had previously been associated with CVD or its risk factors in Caucasians. For five of these regions (CHD: *CDKN2A/CDKN2B*; HDL-C: *FADS1−FADS3*, *PLTP*, *LPL*, and *ABCA1*), the authors were able to use the distinct linkage disequilibrium patterns in African Americans to identify DNA polymorphisms that were more strongly associated with the phenotypes than the previously reported index SNPs found in Caucasian populations. They also developed a new approach for association testing in admixed populations using allelic and local ancestry variation.

GWAS have identified a number of SNPs associated with serum lipid level in populations of European descent. The individual and the cumulative effects of these SNPs on blood lipids are largely unclear in the U.S. population. Chang et al. [64] used data from the second phase of the Third National Health and Nutrition Examination Survey (NHANES III), a nationally representative survey of the U.S. population, to examine associations of 57 GWAS-identified or well-established lipid-related genetic loci with plasma concentrations of HDL-C, LDL-C, TC, and TG. They used multivariable linear regression to examine single SNP associations and the cumulative effect of multiple SNPs on blood lipid levels. Analyses were conducted in adults for each of the three major racial/ethnic groups in the United States: non-Hispanic whites ($n = 2296$), non-Hispanic blacks ($n = 1699$), and Hispanics ($n = 1713$). The allele frequencies of all SNPs varied significantly by race/ethnicity, except rs3764261 in *CETP*. Individual SNPs had small effects on lipid levels, but the effects were consistent across racial/ethnic groups for most SNPs. More GWAS-validated SNPs were replicated in non-Hispanic whites (<67%) than in non-Hispanic blacks (<44%) or Hispanics (<44%). Genetic risk scores were strongly associated with increased lipid levels in each race/ethnic group. The combination of all SNPs into a weighted score explained no more than 11% of the total variance in blood lipid levels. The authors concluded that the combined association of SNPs, used to generate a genetic risk score, was strongly correlated with blood lipid measures in all major race/ethnic groups in the United States and that this knowledge could help in identifying subgroups of patients at high risk for an unfavorable lipid profile.

GWAS were never designed to interrogate actual underlying causal variants; rather, they are used to "tag" the approximate locations of disease variants, typically down to a few hundred kilobases. The development of GWAS methodology may have transformed our ability to discover key genetic factors that are involved in the pathogenesis of CVD, but this only represents the first step in a much longer process. The genes and pathways uncovered using the GWAS approach are likely to be fundamental to disease biology, so it will be crucial to determine how these variants affect the expression and function of the gene products through molecular biology approaches. Therefore, we compiled a list of genes identified by GWAS and additional evidence of their association with lipids. We found 118 loci potentially associated with serum lipid levels that were identified in different GWAS and were also implicated in previously known Mendelian forms of lipid disorders, previously published in candidate gene association studies, or identified through the examination of metabolic phenotypes of knockout mice maintained by the Jackson Laboratory. These genes are described in Table 26.1.

We (JMO and MG-C) identified 118 loci that showed genome-wide significant associations with at least one of the four traits tested (LDL-C, TG, HDLC, and TC). Among these loci, 53 demonstrated genome-wide association with LDL-C, 45 with TG, 56 with HDL-C, and 57 with TC. Only two genes in all GWAS had the same effects on HDL-C—the *LPL* and *CETP* genes. The genes most frequently identified in GWAS for LDL-C were *PCSK9*, *CELSR2*, *APOB*, *ABCG5/8*, *HMGCR*, *LDLR*, *APOE−APOC1−APOC4−APOC2*, and *NSRF*; *CILP2* and *NCAN* were also identified in multiple LDL association studies. The genes most frequently identified in GWAS for TG were *APOB*, *GCKR*, *ANGPTL3−DOCK7−ATG4C*, *MLXIPL*, *LPL*, *FADS1−FADS2−FADS3*, and *APOA−APOC3−APOA4*. The genes most frequently identified in GWAS for HDL-C were *GALNT2*, *LPL*, *ABCA1*, *FADS1−FADS2−FADS3*, *MVK*, *LIPC*, *CETP*, *LCAT*, and *LIPG*. Finally, the genes most frequently identified in GWAS for TC were *CELS42*, *APOB*, *ABCA5G/8*, *LIPG*, and *LDLR* (Table 26.2).

Waterworth et al. [67] conducted a meta-GWAS to identify novel genetic determinants of low LDL-C, HDL-C, and TGs. They combined GWAS data from eight studies, which included up to 17,723 participants and associated information on circulating lipid concentrations. They performed independent replication studies in up to 37,774 participants from eight populations and also in a population of Indian Asian descent. They also assessed the associations between SNPs at lipid loci and CVD risk in up to 9633 cases and 38,684 controls. They identified four novel genetic loci that showed reproducible associations with lipid levels, including a potential functional SNP in the *SLC39A8* gene for HDL-C, an SNP near the *MYLIP/GMPR* and *PPP1R3B* genes for LDL-C, and an

TABLE 26.1 List of Genes Identified in GWAS with Additional Evidence of Association with Lipid Traits

Locus[a]	Chr	Lead SNP	Lead Trait[b]	Other Traits[c]	Alleles/ MAF[d]	Effect Size[e]	*p*	eQTL[f]	CVD[g]	Ethnic[h]	Knockout Mouse Phenotype (Pathway)[i]	Candidate Gene Association[j]	Mendelian Lipid Disorders[k]
LDLRAP1	1	rs12027135	TC	LDL	T/A/0.45	−1.22	4×0^{-11}	Y		+++?	Elevated LDL-C (CMP, SMP)		Familial hypercholesterolemia
PABPC4	1	rs4660293	HDL		A/G/ 0.23	−0.48	4×10^{-10}	Y		++++			
PCSK9	1	rs2479409	LDL	TC	A/G/ 0.30	2.01	2×10^{-28}			++++	Decreased plasma cholesterol (CMP, SMP)	X	Autosomal-dominant hypercholesterolemia
ANGPTL3	1	rs2131925	TG	TC, LDL	T/G/ 0.32	−4.94	9×10^{-43}	Y		++++	Decreased plasma lipids (CMP)		
EVI5	1	rs7515577	TC		A/C/ 0.21	−1.18	3×10^{-8}			+++?			
SORT1	1	rs629301	LDL	TC	T/C/0.22	−5.65	1×10^{-17}	Y	Y	++++			
ZNF648	1	rs1689800	HDL		A/G/ 0.35	−0.47	3×10^{-10}			+++−			
MOSC1	1	rs2642442	TC	LDL	T/C/0.32	−1.39	6×10^{-13}			+++?			
GALNT2	1	rs4846914	HDL	TG	A/G/ 0.40	−0.61	4×10^{-21}			++++			
IRF2BP2	1	rs514230	TC	LDL	T/A/0.48	−1.36	5×10^{-14}			+++?			
TMEM57	1	rs10903129	TC	HDL, LDL, TG	G/A/ 0.54	0.061	5×10^{-10}						
DOCK7	1	rs1167998	TC	TG	A/C/ 0.32	−0.073	6.4×10^{-10}						
ANGPTL3–DOCK7–ATG4C	1	rs1748195	TG	LDL	A/G/ 0.30	−0.08	1.60×10^{-4}						
CELSR2	1	rs646776	LDL	TC	G/T/ 0.21	−0.23	2×10^{-42}						
CR1L	1	rs4844614	LDL		G/A/ 0.32	−0.155	2.19×10^{-12}				Complete lethality during fetal growth and development		
PSRC1	1	rs599839	LDL		A/G/ 0.22	0.174	8.72×10^{-14}						

(*Continued*)

TABLE 26.1 (Continued)

Locus[a]	Chr	Lead SNP	Lead Trait[b]	Other Traits[c]	Alleles/ MAF[d]	Effect Size[e]	*p*	eQTL[f]	CVD[g]	Ethnic[h]	Knockout Mouse Phenotype (Pathway)[i]	Candidate Gene Association[j]	Mendelian Lipid Disorders[k]
APOB	2	rs1367117	LDL	TC	G/A/ 0.30	4.05	4×10^{-114}			+ + + +	Embryonic lethal, heterozygotes have decreased plasma cholesterol, hypobetalipoproteinemia (CMP, LT, RN)	X	Hypobetalipoproteinemia
		rs1042034	TG	HDL	T/C/0.22	−5.99	1×10^{-45}			+ − + +			
GCKR	2	rs1260326	TG	TC	C/T/0.41	8.76	6×10^{-133}	Y		+ + + +	Insulin resistance (CHMP, RN)		
ABCG5/8	2	rs4299376	LDL	TC	T/G/ 0.30	2.75	2×10^{-47}			+ + + +	Sitosterolemia (LT)	X	Sitosterolemia
RAB3GAP1	2	rs7570971	TC		C/A/ 0.34	1.25	2×10^{-8}			+ − ??	Abnormal excitatory postsynaptic currents		
											Abnormal neurotransmitter secretion		
											Enhanced paired pulse facilitation (BD)		
COBLL1	2	rs10195252	TG		T/C/0.40	−2.01	2×10^{-10}	Y		+ + + +			
		rs12328675	HDL		T/C/0.13	0.68	3×10^{-10}			+ + ?+			
IRS1	2	rs2972146	HDL	TG	T/G/ 0.37	0.46	3×10^{-9}	Y	Y	+ + + +	Decreased circulating HDL cholesterol		
											Increased circulating free fatty acids		
											Increased circulating TGs		
											Insulin resistance (ISP)		
R3HDM1	2	rs12465802	LDLC		A/G/ 0.44	0.117	2.63×10^{-8}						
LCT	2	rs2322660	LDL		C/T/0.35	−0.12	2.42×10^{-8}						
MCM6	2	rs309180	LDL		G/A/ 0.36	−0.119	2.43×10^{-8}						
RAF1	3	rs2290159	TC		G/C/ 0.22	−1.42	4×10^{-9}			+ + + ?	Abnormal liver sinusoid morphology (A)		

MSL2L1	3	rs645040	TG		T/G/ 0.22	−2.22	3×10^{-8}			+ + − +			
KLHL8	4	rs442177	TG		T/G/ 0.41	−2.25	9×10^{-12}			+ + + +			
SLC39A8	4	rs13107325	HDL		C/T/0.07	−0.84	7×10^{-11}	Y		+ − ? −			
AFF1	4	rs442177	TG		A/C/ 0.60	0.014	1.5×10^{-7}				Partial postnatal lethality		
ARL15	5	rs6450176	HDL		G/A/ 0.26	−0.49	5×10^{-8}			−??+			
MAP3K1	5	rs9686661	TG		C/T/0.20	2.57	1×10^{-10}			+ + + +	Abnormal heart left ventricle morphology (A)		
HMGCR	5	rs12916	TC	LDL	T/C/0.39	2.84	9×10^{-47}			+ + + ?	Complete embryonic lethality before turning of embryo (A, CMP)	X	
TIMD4	5	rs6882076	TC	LDL, TG	C/T/0.35	−1.98	7×10^{-28}			+ + + ?			
C5ORT35	5	rs6867983	TG		C/T/0.14	0.014	6.1×10^{-6}						
RNF130	5	rs13161895	LDL		C/T/0.08	0.151	2.3×10^{-5}						
MYLIP	6	rs3757354	LDL	TC	C/T/0.22	−1.43	1×10^{-11}			+ − − +			
HFE	6	rs1800562	LDL	TC	G/A/ 0.06	−2.22	6×10^{-10}			+ + ? +			
HLA	6	rs3177928	TC	LDL	G/A/ 0.16	2.31	4×10^{-19}	Y		+ + + ?			
		rs2247056	TG		C/T/0.25	−2.99	2×10^{-15}			+ + + −			
C6orf106	6	rs2814944	HDL		G/A/ 0.16	−0.49	4×10^{-9}	Y		+ + + −			
		rs2814982	TC		C/T/0.11	−1.86	5×10^{-11}		Y	− − + ?			
FRK	6	rs9488822	TC	LDL	A/T/0.35	−1.18	2×10^{-10}	Y		+ + + ?	Decreased circulating triiodothyronine (RPT)		
CITED2	6	rs605066	HDL		T/C/0.42	−0.39	3×10^{-8}			+ + − +	Abnormal heart development (RES)		
LPA	6	rs1564348	LDL	TC	T/C/0.17	−0.56	2×10^{-17}		Y	+ + ? +	Decreased total body fat, hemorrhage		

(*Continued*)

TABLE 26.1 (Continued)

Locus[a]	Chr	Lead SNP	Lead Trait[b]	Other Traits[c]	Alleles/ MAF[d]	Effect Size[e]	*p*	eQTL[f]	CVD[g]	Ethnic[h]	Knockout Mouse Phenotype (Pathway)[i]	Candidate Gene Association[j]	Mendelian Lipid Disorders[k]
		rs1084651	HDL		G/A/ 0.16	1.95	3×10^{-8}			++?+			
IGF2R	6	rs456598	LDL		A/G/ 0.87	−0.015	8.4×10^{-7}				Complete prenatal lethality (A, RAMP)		
DNAH11	7	rs12670798	TC	LDL	T/C/0.23	1.43	9×10^{-10}			+++?	Abnormal heart morphology (AG)		
NPC1L1	7	rs2072183	TC	LDL	G/C/ 0.25	2.01	3×10^{-11}			+−+?	Increased cholesterol level (CMP, LT, STMP)		
TYW1B	7	rs13238203	TG		C/T/0.04	−7.91	1×10^{-9}			+???			
MLXIPL	7	rs17145738	TG	HDL	C//T/ 0.12	−9.32	6×10^{-58}	Y		++++	Decreased circulating cholesterol and free fatty acids (GL)		
KLF14	7	rs4731702	HDL		C/T/0.48	0.59	1×10^{-15}		Y	++++			
PPP1R3B	8	rs9987289	HDL	TC, LDL	G/A/ 0.09	−1.21	6×10^{-25}	Y		++++			
PINX1	8	rs11776767	TG		G/C/ 0.37	2.01	1×10^{-8}			−+++	Complete embryonic lethality during organogenesis (MMPC)		
NAT2	8	rs1495741	TG	TC	A/G/ 0.22	2.85	5×10^{-14}		Y	−+++	Abnormal xenobiotic pharmacokinetics		
LPL	8	rs12678919	TG	HDL	A/G/ 0.12	−13.64	2×10^{-115}		Y	++++	Decreased circulating HDL-C and LDL-C, increased circulating TG and VLDL (LMP)	X	LPL deficiency
CYP7A1	8	rs2081687	TC	LDL	C/T/0.35	1.23	2×10^{-12}			+++?	Nearly absent cholesterol absorption (CMP, STMP)	X	Hypercholesterolemia
TRPS1	8	rs2293889	HDL		G/T/ 0.41	−0.44	6×10^{-11}			++++	Complete neonatal lethality (RPT)		Trichorhinophalangeal syndrome, type 1
		rs2737229	TC		A/C/ 0.30	−1.11	2×10^{-8}			++−?			
TRIB1	8	rs2954029	TG	TC, LDL, HDL	A/T/0.47	−5.64	3×10^{-55}		Y	++++			
PLEC1	8	rs11136341	LDL	TC	A/G/ 0.40	1.4	4×10^{-13}			++++	Abnormal heart morphology (RN)		
XKR6–AMAC1L2	8	rs7819412	TG		A/G/ 0.48	−0.04	3×10^{-8}						

TTC39B	9	rs581080	HDL	TC	C/G/0.18	−0.65	3×10^{-12}			+ − + +			
ABCA1	9	rs1883025	HDL	TC	C/T/0.25	−0.94	2×10^{-33}			+ + + +	Nearly absent HDL-C (LT, CMP)	X	Tangier disease
AB0	9	rs9411489	LDL	TC	C/T/0.20	2.24	6×10^{-13}		Y	????			
JMJD1C	10	rs10761731	TG		A/T/0.43	−2.38	3×10^{-12}			+ + + +			
CYP26A1	10	rs2068888	TG		G/A/0.46	−2.28	2×10^{-8}			+ + + +	Abnormal heart morphology (RAMP)		
GPAM	10	rs2255141	TC	LDL	G/A/0.30	1.14	2×10^{-10}			+ + + ?	Decreased circulating insulin, cholesterol, and TG and decreased liver TGs (FAMP)		
AMPD3	11	rs2923084	HDL		A/G/0.17	−0.41	5×10^{-8}			+ + − +			
SPTY2D1	11	rs10128711	TC		C/T/0.28	−1.04	3×10^{-8}	Y		+ − + ?			
LRP4	11	rs3136441	HDL		T/C/0.15	0.78	3×10^{-18}	Y		+ + + ?	Abnormal postnatal growth (incisor and limbs) (RPT)		
FADS1 − 2−3	11	rs174546	TG	HDL, TC, LDL	C/T/0.34	3.82	5×10^{-24}	Y		+ + + +	Increased liver TG (FAMP, LT)		
APOA1	11	rs964184	TG	TC, HDL, LDL	C/G/0.13	16.95	7×10^{-240}		Y	+ + + +	Decreased cholesterol efflux and increased circulating TG (CMP, LT)	X	ApoA-1 deficiency
UBASH3B	11	rs7941030	TC	HDL	T/C/0.38	0.97	2×10^{-10}			+ + + ?			
ST3GAL4	11	rs11220462	LDL	TC	G/A/0.14	1.95	1×10^{-15}	Y		+ + + +	Abnormal platelet physiology		
APOA1/ APOC3/ APOA4	11	rs12277004	TG	LDL, TC	C/C/0.93	−0.181	5.4×10^{-13}				Increased susceptibility to atherosclerosis	X	
MADD− FOLDH	11	rs7395662	HDL	LDC, TC, TG	A/G/0.61	−0.073	6.0×10^{-11}				Decreased circulating HDL and cholesterol		
NR1H3	11	rs2167079	HDL		G/A/0.41	0.04	5.13×10^{-8}						
PDE3A	12	rs7134375	HDL		C/A/0.42	0.4	4×10^{-8}			+ + + +	Female infertility (RES)		

(*Continued*)

TABLE 26.1 (Continued)

Locus[a]	Chr	Lead SNP	Lead Trait[b]	Other Traits[c]	Alleles/ MAF[d]	Effect Size[e]	*p*	eQTL[f]	CVD[g]	Ethnic[h]	Knockout Mouse Phenotype (Pathway)[i]	Candidate Gene Association[j]	Mendelian Lipid Disorders[k]
LRP1	12	rs11613352	TG	HDL	C/T/0.23	−2.7	4×10^{-10}			+ + ?+	Complete embryonic lethality during organogenesis (A, LT)		
MVK	12	rs8134504	HDL		T/C/0.47	−0.44	7×10^{-15}	Y		+ + ?+			
BRAP	12	rs11065987	TC	LDL	A/G/ 0.42	−0.96	7×10^{-12}			+ + ??			
HNF1A	12	rs1169288	TC	LDL	A/C/ 0.33	1.42	1×10^{-14}		Y	+ + + ?	Abnormal pancreatic islet morphology and increased cholesterol (CMP, FAMP, HIR)		
SBNO1	12	rs4759375	HDL		C/T/0.06	0.86	7×10^{-9}			+ ??+			
ZNF664	12	rs4765127	HDL	TG	G/T/ 0.34	0.44	3×10^{-10}			−+ − +			
SCARB1	12	rs838880	HDL		T/C/0.31	0.61	3×10^{-14}			+ + − ?	Increased susceptibility to atherosclerosis and increased circulating cholesterol (CMP, LT)	X	
MMAB	12	rs2338104	HDL		G/C/ 0.45	−0.07	1×10^{-10}						
OSBPL8/ ZDHHC17	12	rs17259942	HDL		A/G/ 0.12	0.168	8.61×10^{-8}						
NYNRIN	14	rs8017377	LDL		G/A/ 0.47	1.14	5×10^{-11}			+ − + +			
CAPN3	15	rs2412710	TG		G/A/ 0.02	7	2×10^{-8}			+ ?? −	Dilated cardiomyopathy (RES)		Muscular dystrophy, Limb−Girdle, type 2A: LGMD2A
FRMD5	15	rs2929282	TG		A/T/0.05	5.13	2×10^{-11}	Y		+ − − − −			
LIPC	15	rs1532085	HDL	TC, TG	G/A/ 0.39	1.45	3×10^{-96}	Y		+ + + +	Increased circulating TG, HDL cholesterol and LDL cholesterol (CMP, FAMP, LMP)	X	Hepatic lipase deficiency
LACTB	15	rs2652834	HDL		G/A/ 0.20	−0.39	9×10^{-9}	Y		+ ???			
GCOM1	15	rs937254	HDL		G/A/ 0.57	0.077	5.4×10^{-6}						
CTF1	16	rs11649653	TG		C/G/ 0.40	−2.13	3×10^{-8}	Y		+ ?? −	Motor neuron degeneration (BD)		

CETP	16	rs3764261	HDL	TC, LDL, TG	C/A/ 0.32	3.39	7×10^{-380}			+ + + +	Decreased cholesterol and TG (involves transgenes)		CETP deficiency
LCAT	16	rs16942887	HDL		G/A/ 0.12	1.27	8×10^{-33}	Y		+ + + +	Decreased circulating HDL cholesterol (CMP, LMP, STMP)		LCAT deficiency (very low HDL)
HPR	16	rs2000999	TC	LDL	G/A/ 0.20	2.34	3×10^{-24}			+ + + ?			
CMIP	16	rs2925979	HDL		C/T/0.30	−0.45	2×10^{-11}			+ + + +			
CTCF–PRMTB	16	rs2271293	HDL		A/G/ 0.87	−0.129	8.3×10^{-16}						
STARD3	17	rs11869286	HDL		C/G/ 0.34	−0.48	1×10^{-13}	Y		+ + + +	Increased liver cholesterol (LT, STMP)		
OSBPL7	17	rs7206971	LDL	TC	G/A/ 0.49	0.78	2×10^{-8}	Y		+ + − +			
ABCA8	17	rs4148008	HDL		C/G/ 0.32	−0.42	2×10^{-10}			+ + + +			
PGS1	17	rs4129767	HDL		A/G/ 0.49	−0.39	8×10^{-9}			+ + + +			
LIPG	18	rs7241918	HDL	TC	T/G/ 0.17	−1.31	3×10^{-49}	Y		+ + + +	Abnormal vascular endothelial cell physiology and increased circulating HDL cholesterol (LMP)		
MC4R	18	rs12967135	HDL		G/A/ 0.23	−0.42	7×10^{-9}			+ + + +	Increased circulating leptin and hyperglycemia (HIR)		
ANGPTL4	19	rs7255436	HDL		A/C/ 0.47	−0.45	3×10^{-8}	Y		+ + + +	Decreased circulating cholesterol and TG (A)		
LDLR	19	rs6511720	LDL	TC	G/T/ 0.11	−6.99	4×10^{-117}		Y	+ + ?+	Elevated LDL-C (LT, CMP, LMP, STMP)	X	Homozygous familial hypercholesterolemia
LOC55908	19	rs737337	HDL		T/C/0.08	−0.64	3×10^{-9}			+ + + +			
CILP2	19	rs10401969	TC	TG, LDL	T/C/0.07	−4.74	3×10^{-38}		Y	+ + + ?			
APOE	19	rs4420638	LDL	TC, HDL	A/G/ 0.17	7.14	9×10^{-147}		Y	+ + + +	Hypercholesterolemia and atherosclerotic lesions (LT, CMP, LMP, RN)	X	Familial dysbetalipoproteinemia (elevated chylomicrons and VLDL remnants)
		rs439401	TG		C/T/0.36	−5.5	1×10^{-30}	Y		+ + + ?			

(*Continued*)

TABLE 26.1 (Continued)

Locus[a]	Chr	Lead SNP	Lead Trait[b]	Other Traits[c]	Alleles/ MAF[d]	Effect Size[e]	*p*	eQTL[f]	CVD[g]	Ethnic[h]	Knockout Mouse Phenotype (Pathway)[i]	Candidate Gene Association[j]	Mendelian Lipid Disorders[k]
FLJ36070	19	rs492602	TC		A/G/ 0.49	1.27	2×10^{-10}			+ − +?			
LILRA3	19	rs386000	HDL		G/C/ 0.20	0.83	4×10^{-16}	Y		+ − + −			
NCAN	19	rs2304130	TC	TG, LDL	A/G/ 0.07	−0.153	2.0×10^{-15}						
TOMM40 APOE	19	rs157580	TC	TG	A/G/ 0.33	−0.09	5.1×10^{-17}						
APOE–APOC1–APOC4–APOC2	19	rs4420638	LDL		A/G/ 0.16	0.29	4×10^{-27}						
ERGIC3	20	rs2277862	TC		C/T/0.15	−1.19	4×10^{-10}	Y		+ + +?			
MAFB	20	rs2902940	TC	LDL	A/G/ 0.29	−1.38	6×10^{-11}			− −+?	Complete neonatal lethality (DNAD, BD)		
TOP1	20	rs6029526	LDL	TC	T/A/0.47	1.39	4×10^{-19}	Y		+ + + +	Complete embryonic lethality before implantation (DNAI)		
HNF4A	20	rs1800961	HDL	TC	C/T/0.03	−1.88	1×10^{-15}			+ + + −	Complete embryonic lethality (LMP, DNAD, RN, STMP)		
PLTP	20	rs6065906	HDL	TG	T/C/0.18	−0.93	2×10^{-22}			+ − + +	Decreased circulating HDL cholesterol and increased LDL and VLDL-C (LT)		
UBE2L3	22	rs181362	HDL		C/T/0.20	−0.46	1×10^{-8}	Y		+ + + +			
PLA2G6	22	rs5756931	TG		T/C/0.40	−1.54	4×10^{-8}			+??+	Neurodegeneration with brain iron accumulation 2A: NIA2A (LMP)		

[a]*Locus: either a plausible biological candidate gene at the identified locus or the annotated gene closest to the lead SNP.*
[b]*Lead trait: the lipid trait with best* p *value among all four traits.*
[c]*Other traits: additional lipid traits with* $p < 5 \times 10^{-9}$.
[d]*Alleles/MAF: the major allele, minor allele, and minor allele frequency (MAF) within the combined cohort.*
[e]*Effect size: mg/dL for the lead trait, modeled as an additive effect of the minor allele.* p *values are listed for the lead trait.*
[f]*In the eQTL column, "Y" indicates that the lead SNP has an eQTL with at least one gene within 500 kb, with* $p < 5 \times 10^{-8}$ *in at least one of the three tissues tested (liver, omental fat, and subcutaneous fat).*
[g]*In the CVD column, "Y" indicates that the lead SNP meets the prespecified statistical significance threshold of* $p < 0.001$ *for association with CVD and concordance between the direction of lipid effect and the change in CVD risk.*
[h]*In the "ethnic" column, "+ " indicates a concordant effect of the variant between the primary meta-analysis cohort and the European or non-European group, " − " indicates a discordant effect on the lead trait, and "?" indicates data not available for the group. In order, the ethnic groups are European, East Asian, South Asian, and African American [62].*
[i]*Knockout mouse phenotype: gene examined in regard to metabolic phenotypes and pathways (xx) of knockout mice maintained by the Jackson laboratory. Pathways: A, apoptosis; AG, axon guidance; BD, brain development; CMP, cholesterol metabolic process; Gl, glycolysis; HIR, humoral immune response; ISP, insulin signaling pathway; JNK, JNK cascade; LMP, lipid metabolic process; LT, lipid transport; MIT, metal ion transport; MMPC, mitotic metaphase plate congression; PPT, regulation of protein transport; RES, response to extracellular stimulus; RN, response to nutrient; RNL, response to nutrient levels; SMP, sterol metabolic process.*
[j]*Candidate gene association: gene examined in previously published candidate gene association studies.*
[k]*Mendelian lipid disorders: literature survey of previously known Mendelian form of lipid disorders [65]. Chr, chromosome.*

TABLE 26.2 Replication of Genetic Associations with Lipid Levels Across Public GWASs

Locus[a]	Chr	LDL-C	TG	HDL-C	TC
LDLRAP1	1	1	1		
PABPC4	1			1	
PCSK9	1	1, 3, 4, 5, 6, 8			1
ANGPTL3	1	1	1, 3, 4		1
EVI5	1				1
SORT1	1	1, 4			1
ZNF648	1			1	
MOSC1	1	1			1
GALNT2	1		1, 4, 8	1, 3, 4, 5, 6, 8	
IRF2BP2	1	1			1
TM3M57	1	2	2	2	2
ANGPTL3–DOCK7–ATG4C	1	6, 8	2, 3, 4, 5, 8		8
CELSR2	1	2, 3, 4, 5, 6, 8			2, 7, 8
CR1L	1	4			
PSRC1	1	7, 8			
APOB	2	1, 3, 4, 5, 6, 8	1, 2, 3, 4, 5, 8	1, 2, 5	1, 2, 8
GCKR	2	8	1, 2, 3, 4, 5, 8		1
ABCG5/8	2	1, 2, 3, 7, 8	2	2	1, 2, 8
RAB3GAP1	2	7			1
COBLL1	2		1		
IRS1	2		1	1	
R3HDM1	2	7			
LCT	2	7			
MCM6	2	7			
RAF1	3				1
MSL2L1	3		1		
KLHL8	4		1		
SLC39A8	4			1, 5	
AFF1	4		5		
ARL15	5			1	
MAP3K1	5		1		
HMGCR	5	1, 2, 3, 4, 5, 8			1, 2
TIMD4	5	1, 3, 8	1		1
C5ORT35	5		5		
RNF130	5	6			
MYLIP	6	1, 5			1
HFE	6	1			1
HLA	6	1	1		1

(*Continued*)

TABLE 26.2 (Continued)

Locus[a]	Chr	LDL-C	TG	HDL-C	TC
C6orf106	6			1	1
FRK	6	1			1
CITED2	6			1	
LPA	6	1		1	1
IGF2R	6	5			
DNAH11	7	1, 2	2	2	1, 2
NPC1L1	7	1			1
TYW1B	7		1		
MLXIPL	7		1, 2, 3, 4, 5, 8	1	
KLF14	7			1	
PPP1R3B	8	1, 5		1, 6	1
PINX1	8		1		
NAT2	8		1		1
LPL	8		1, 2, 3, 4, 5, 7, 8	1, 2, 3, 4, 5, 6, 7, 8	
CYP7A1	8	1			1
TRPS1	8			1	
TRIB1	8	1, 5	1, 3, 4, 8	1	1, 2
PLEC1	8	1			1
XKR6–AMAC1L2	8		3		
TTC39B	9			1, 3, 5, 8	1
ABCA1	9			1, 2, 3, 4, 5, 6, 8	1, 2
AB0	9	1			1
JMJD1C	10		1		
CYP26A1	10		1		
GPAM	10	1			1
AMPD3	11			1	
SPTY2D1	11				1
LRP4	11			1	
FADS1-2–3	11	1, 2, 4	1, 2, 3, 5, 8	1, 2, 3, 5, 6, 8	1, 2
APOA1/APOC3/APOA4	11	5	2, 3, 4, 5, 8	3, 4, 5, 8	
APOA1	11	1	1	1	1
UBASH3B	11			1	1
ST3GAL4	11	1			1
MADD–FOLH1	11	2	2	2	2
NR1H3	11			4	
PDE3A	12			1	
LRP1	12		1	1	
MVK	12			1, 3, 4, 5, 8	

(*Continued*)

TABLE 26.2 (Continued)

Locus[a]	Chr	LDL-C	TG	HDL-C	TC
BRAP	12	1			1
HNF1A	12	1, 3, 8			1
SBNO1	12			1	
ZNF664	12		1	1	
SCARB1	12			1	
MMAB	12			3, 4, 5, 8	
OSBPL8–ZDHHC17	12			7	
NYNRIN	14	1			
CAPN3	15		1		
FRMD5	15		1		
LIPC	15		1	1, 2, 3, 4, 5, 6, 8	1, 2
LACTB	15			1	
GCOM1	15			6	
CTF1	16		1		
CETP	16	1, 2, 8	1, 2, 5	1, 2,3, 4, 5, 6, 7, 8	1
LCAT	16			1, 3, 4, 5, 6, 8	
HPR	16	1			1
CMIP	16			1	
CTCF–PRMT8	16			2	
STARD3	17			1	
OSBPL7	17	1			1
ABCA8	17			1	
PGS1	17			1	
LIPG	18			1, 2, 3, 4, 5, 7, 8	1, 2, 8
MC4R	18			1	
ANGPTL4	19			1, 3, 8	
LDLR	19	1, 2, 3, 4, 5, 6, 8			1, 2, 8
LOC55908	19			1	
CILP2	19	1, 3, 4, 5	1, 3, 5, 8		1
APOE	19	1, 8	1	1	1, 8
FLJ36070	19				1
LILRA3	19			1	
NCAN	19	2, 3, 4, 8	2, 3, 8		2
TOMM40–APOE	19	2	2, 8		2, 8
APOE–APOC1–APOC4–APOC2	19	3, 4, 5, 6, 8	8		8
ERGIC3	20				1
MAFB	20	1, 3, 8			1
TOP1	20	1			1

(*Continued*)

TABLE 26.2 (Continued)

Locus[a]	Chr	LDL-C	TG	HDL-C	TC
HNF4A	20			1, 3, 8	1
PLTP	20		1, 3, 8	1, 3, 6, 8	
UBE2L3	22			1	
PLA2G6	22		1		

[a]*Each gene listed in the "locus" column is either a plausible biological candidate gene in the locus or the nearest annotated gene to the lead SNP.*
Chr, chromosome; GWAS: 1, Teslovich et al. [62]; 2, Aulchenko et al. [59]; 3, Kathiresan et al. [60]; 4, Sabbatti et al. [58]; 5, Waterworth et al. [67]; 6, Lettre et al. [63]; 7, Ma et al. [61]; and 8, Chang et al. [64].

SNP at the *AFF1* gene for TGs. SNPs at the *CELSR2−APOB−APOE−APOC1−APOC4−APOC2* cluster, *LPL−ZNF259−APOA5−APOA4−APOC3−APOA1* cluster, and *TRIB1* locus showed strong statistical association with one or more lipid traits and were also associated with CVD risk.

Most recently the Global Lipids Genetics Consortium [66], in a worldwide meta-analysis expanded to 188,577 European individuals genotyped either on GWAS or specialized Metabochip arrays, identified and annotated 157 loci associated with lipid levels at genome-wide significance ($p < 5 \times 10^{-8}$), including 62 not previously reported. Diverse (European, East Asian, South Asian, and African ancestry) populations comprising 7898 were used to narrow the signals in 12 loci. Of the 62 novel loci, which identified 240 genes within 100 kb, the role of 38 in lipid regulation was supported by previous evidence as reported in the literature or curated pathway databases, and the other 24 had no such evidence. Several other analyses were performed to gain insight into the role of these variants, including analysis of the relationship with CAD risk and lipid subfractions, providing evidence that LDL-C and TG but not HDL-C levels are causally related to CAD risk [66]. Because of the complexity of the analyses and combination of platforms used, these results have not been included in Tables 26.1 and 26.2.

These increasingly large GWAS studies and meta-analyses have made possible the identification of numerous common variants with increasingly smaller effect sizes that were not investigated by previous candidate gene studies. Ultimately, one of the major challenges going forward will be to determine how these recently uncovered variants affect the expression and function of gene products using molecular biology approaches. Detailed genotype−phenotype studies will be required to determine the mechanisms involved and how these loci potentially interact with and are involved in other disorders. Only by continuing to uncover the functional context of these genetic variants and understanding their interactions can these findings be truly translated into meaningful benefits for patient care. Further understanding of the effects of low frequency and rare variants in these and other genes likely to have larger effects will also be important for clarifying the genetic contributions to lipid level variation. As GWAS generally only tests for common variants, such large effect variants have not been accessible in population studies. Identifying these will require large, high-throughput sequencing efforts involving thousands of DNA samples, which is becoming increasingly possible with the advent of next-generation sequencing technologies and decreasing costs. Studying founder populations, in which frequencies of otherwise rare variants can be dramatically increased, also offers the opportunity to be able to characterize phenotypic effects of rare variants.

A Founder Populations

While GWAS in outbred populations are generally not powered to detect rare variant associations, the founder effect enables a rare variant to reach a high frequency in a closed population such that it can be tagged by a common variant at the lower end of the frequency spectrum. In 2008, we (TIP and colleagues) [68] performed a GWAS for TG levels before and after a high fat meal in 806 Pennsylvania Old Order Amish individuals. An SNP on the array near the *APOA1/C3/A4/A5* region showed genome-wide significant association with lower fasting ($p = 4 \times 10^{-14}$) and postprandial ($p = 2.8 \times 10^{-10}$) TG levels. Sequencing of the APOC3 gene revealed a previously unreported loss-of-function mutation R19X to be present in approximately 5% of the Amish population. In addition to being associated with a cardioprotective lipid profile including low TG, high HDL-C, and low LDL-C, the variant was also associated with less coronary artery calcification, a subclinical measure of CVD. [68] Interestingly, we also found that the Amish also have a dramatically elevated carrier frequency (12%) of an otherwise rare APOB variant R3527Q causing familial hypercholesterolemia; our large sample of carriers showed that

mutation carriers had more coronary calcification even after adjusting for LDL-C levels, suggesting an effect of lifelong exposure to elevated LDL-C and/or other mediating factors [69] and on a practical level suggesting that genetic testing may offer benefits for prediction and management of CVD risk beyond cholesterol level measurement.

B High Throughput Sequencing

In 2014, The Exome Sequencing Project [70] reported on efforts to better understand the role of rare variants in the population in TG levels and cardiovascular risk. They sequenced protein coding regions of 18,666 genes in 3734 individuals with European or African ancestry and analyzed the association of groups of rare variants within each gene with TG levels. The gene most strongly associated with TG levels was *APOC3*, resulting primarily from four mutations, including R19X, found in five or more individuals each. Genotyping these variants in 41,671 additional European/African ancestry participants yielded association with 39% lower TG, higher HDL-C and a trend toward lower LDL-C. Importantly, genotyping these four in 110,970 individuals revealed that carriers, comprising 1/150 individuals, had a 40% lower risk of coronary heart disease and no evidence of increased hepatic fat, providing clinical confirmation that loss of apoC-III is cardioprotective. A separate study reported at the same time by Jørgensen et al. [71] confirmed these findings: in 75,725 individuals, sequencing of the coding and flanking regions of *APOC3* was conducted and three LOF *APOC3* mutations were found in 1/290 individuals and were associated with 44% lower TG levels and 41% and 36% lower risk of ischemic vascular disease and ischemic heart disease, respectively [71]. Together these findings along with previous findings regarding apoC-III function have helped to establish a primary role for TG variation in CVD etiology. Further studies of individuals with these variants is underway and expected to increase our understanding of the etiology of CVD and specifically increase our understanding of apoC-III function; meanwhile, clinical trials of strategies to reduce CVD risk by directly interfering with apoC-III production are already underway [72] and may offer improvements in CVD prevention over existing therapies which act in part through apoC-III. In a similar manner, gain of function mutations in *PCSK9*, encoding an inhibitor of the LDL receptor, were first identified as a cause of familial hypercholesterolemia (elevated LDL-C) through a linkage and fine mapping study in families [73]; subsequently, studies of individuals with very low LDL-C levels revealed cardioprotective loss-of-function mutations reducing LDL-C and CVD risk in 2% of African Americans and $<1\%$ of European Americans [74] that have led to the development of commercially available *PCSK9* inhibitors to treat hypercholesterolemia [75,76].

III DEVELOPMENT OF CARDIOVASCULAR SCORE

Measures of cardiovascular risk, such as the Framingham score, have long been used in public health research and practice [77–80]. These risk scores play an important role in screening programs to identify susceptible individuals before the onset of clinical symptoms and facilitate primary prevention [81]. Cardiovascular risk scores are also used in epidemiological research, namely as measures of exposure, as stratification variables, or as measures of potential confounders [82,83].

The predictive ability of existing cardiovascular risk scores varies greatly between populations and is particularly low for ethnic minorities [84]. This variability merits further investigation; most current cardiovascular risk measures are based on cohorts from high-income Western countries [85,86], and their generalizability to populations from low- or middle-income countries or ethnically and economically diverse communities in high-income countries is limited.

In addition, low LDL-C and high HDL-C, TG, and TC concentrations are important and heritable risk factors for CVD. Although GWAS of circulating lipid levels have identified numerous disease-associated loci, a substantial portion of the heritability of these traits remains unexplained. Evidence of unexplained genetic variance can be detected by combining multiple independent markers into additive genetic risk scores. These polygenic scores, which were constructed using results from the ENGAGE Consortium GWAS on serum lipids [59], were used to predict lipid levels in an independent population-based study, the Rotterdam Study II. This study also tested for evidence of a shared genetic basis for different lipid phenotypes. The polygenic score approach was used to identify an alternative genome-wide significance threshold for subsequent pathway analysis, and the results of that analysis were compared with those based on the classical genome-wide significance threshold. Demirkan et al. [87] provide evidence suggesting that many loci that influence circulating lipid levels remain undiscovered. Cross-prediction models suggested a small overlap between the polygenic backgrounds involved in determining LDL-C, HDL-C, and TG levels. Pathway analysis utilizing the best polygenic score for TC uncovered extra information compared with a model that used only genome-wide significant loci.

Using prediction modeling, Demirkan et al. [87] explain up to 4.8% of the variance in HDL-C, 2.6% in LDL-C, 3.8% in TG, and 2.7% in TC. However, these proportions are much lower than those identified by the Global Lipid Genetics Consortium, which were estimated to explain 12.4% (TC), 12.2% (LDL-C), 12.1% (HDL-C), and 9.6% (TG) of the variance in the FHS sample, as reported by

Teslovich et al. [62]. This is expected because increases in sample size lead to better estimation of SNP effect sizes.

GWAS have yielded a wealth of information about the pathogenesis of dyslipidemia. Even as we continue to use these studies to uncover novel protein and gene variants involved in lipoprotein metabolism, a significant investment of effort and resources needs to be made to fully exploit the findings of these important studies. Further research facilitated by availability of large-scale high throughput genome-wide sequencing as underway in the T2D-Genes Consortium, including in founder populations, is expected to include the identification of additional less frequent variants with large effects, which may help us narrow down the genetic loci of interest and provide proof-of-concept of the potential benefits of modulating the functions of these proteins as in the case of *PCSK9* [75,76,88] and *APOC3* [68,70–72].

The GWAS and sequencing studies reviewed here represent the culmination of years of groundwork, but the studies and results reported in this chapter only scratch the surface of the true potential of this approach. In addition to the obvious next steps of replication, signal refinement, and identification of additional causative variants, the definition of the biological roles of GWAS- and other identified genes in metabolic pathways will require substantial further research, including studies of these genes in model systems.

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Chapter 27

The Role of Diet in the Prevention and Treatment of Cardiovascular Disease

Chesney Richter[1], Ann Skulas-Ray[1] and Penny Kris-Etherton[2]
[1]The University of Arizona, Tucson, AZ, United States, [2]Pennsylvania State University, State College, PA, United States

I INTRODUCTION

Cardiovascular disease (CVD) still remains the leading cause of death in the United States despite steady declines in prevalence due to decades of research and many advances in pharmacological and surgical treatments [1]. Health care costs for CVD in the United States were estimated to be over $315 billion in 2011−12 (including both direct medical costs and indirect costs, such as lost work days and productivity) [1], and are expected to exceed $1 trillion annually by 2030 [2]. Thus, there remains a need for effective CVD prevention strategies and improvements in current standard-of-care pharmaceutical interventions to reduce CVD risk. CVD risk factors are characterized as nonmodifiable (e.g., age, ethnicity, sex, and family history) or modifiable [3]. The traditional modifiable risk factors include elevated total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and hypertension. It is also generally agreed upon that elevated triglycerides (TGs), reduced high-density lipoprotein-cholesterol (HDL-C), type 2 diabetes, and overweight/obesity also increase CVD risk [4,5]. Most interventions target the traditional modifiable risk factors, but it is important to note that other factors also contribute to the development of CVD. For instance, it has been estimated that determining risk solely on cholesterol screening would fail to identify approximately half of the 1.3 million individuals who develop myocardial infarction (MI) each year [6]. There is growing evidence to support the role of new and emerging CVD risk factors, including inflammation, oxidative stress, the omega-3 index, central blood pressure, arterial stiffness, and postprandial derangements in metabolism or altered lipoprotein properties (e.g., particle size) that are not assessed in a standard lipid panel. Both traditional and emerging risk factors can be modified by dietary and/or lifestyle changes, and a healthy diet has long been a cornerstone for the management of CVD risk factors.

The American Heart Association (AHA) and other organizations, such as the U.S. Department of Agriculture (USDA), U.S. Department of Health and Human Services, and Academy of Nutrition and Dietetics (AND) regularly issue diet and lifestyle recommendations to reduce chronic disease risk, including (or specific to) CVD. Historically, these dietary recommendations have focused on macronutrient targets (e.g., <7−10% of calories from saturated fat). Recently, recommendations have shifted toward a whole food, dietary pattern approach. This change is evident in the 2015−2020 Dietary Guidelines for Americans (2015−2020 DGA) and the 2013 American Heart Association/American College of Cardiology (AHA/ACC) Guideline on Lifestyle Management to Reduce Cardiovascular Risk [7,8]. Thus, this chapter briefly reviews the current dietary guidelines for minimizing CVD risk, and discusses the role of both macronutrients and evidence-based dietary patterns in preventing and treating CVD.

A Dietary Guidelines and Recommendations

The 2015−2020 DGA [7] and the 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk [8] are the most recent dietary guidelines for reducing the risk of CVD (and other chronic diseases). The 2015−2020 DGA encourages the consumption of nutrient-dense foods, including a variety of vegetables, fruits (especially whole), grains (with at least

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half being whole grains), fat-free or low-fat dairy, oils, and a variety of protein foods (e.g., seafood, lean meats, eggs, legumes, nuts, seeds, and soy products) [7]. Foods high in saturated fatty acid (SFA), *trans* fatty acid (TFA), added sugars, and sodium should be limited. The recommended intakes of nutrient-dense foods that are associated with reduced CVD risk—when not exceeding energy needs—are presented in Table 27.1. The 2015−2020 DGA recommends consuming <10% of calories from SFA [7]. The AHA/ACC Guideline recommends an even greater reduction to 5−6% of calories [8]. Both recommend that TFA consumption be limited [7,8]. For adults who choose to consume alcohol, consumption should be limited to one drink per day for women and up to two drinks per day for men [7]. The calorie content of alcohol should also be considered so as to ensure that daily energy needs are not exceeded. The 2015−2020 DGA recommends that sodium intake be limited to <2300 mg/day [7]. The AHA/ACC Guideline further specifies that sodium intake be reduced by at least 1000 mg/day and that intake should not exceed 2400 mg/day, with a sodium intake of 1500 mg/day being optimal [8]. Although these recommendations are based on current evidence, our understanding of how macronutrients, micronutrients, and other dietary bioactives affect CVD risk is a continually evolving process; thus, it is likely that dietary approaches for reducing CVD risk will also continue to advance and become even more effective as our knowledge evolves.

II EFFECTS OF MACRONUTRIENTS ON CVD RISK FACTORS

Different macronutrients have very specific effects on CVD risk factors; thus, although current dietary guidelines for reducing CVD risk now place greater emphasis on the overall dietary pattern, target ranges for macronutrient intakes remain an important element of nutrition interventions. Historically, nutrition recommendations have focused on dietary fat and reducing SFA and TFA intake. Recently, more attention has been directed toward the effect of modifying the type and/or amount of carbohydrates (CHO) and protein, as well. For instance, the importance of whole grains versus refined CHO are now well recognized. Potential differences between the effects of dietary protein from plant versus animal foods on CVD risk have also generated much interest. The following sections discuss each macronutrient and their effects on CVD risk.

A Dietary Fat

The role of dietary fat in the onset and progression of CVD has been studied extensively, and determining the ideal quantity and quality of dietary fat is an important consideration for CVD prevention and treatment. Table 27.2 summarizes the dietary fat recommendations issued by several expert organizations, as well as the average daily intake of the U.S. population. The 2015−2020 DGA [7], the joint report by the Food and Agriculture Organization and the World Health Organization [12], and the AND [13] all recommend 20−35% of energy from fat but differ with regard to specific fatty acid (FA) recommendations. However, the recommendation to restrict total fat intake is an area of controversy, and may not be as beneficial for reducing chronic disease risk as it was once thought. For instance, in the Women's Health Initiative Dietary Modification Trial [14], there was no reduction in the risk of CVD (including specifically coronary heart disease [CHD] and stroke) in women instructed to follow a low-fat diet (20% of calories from fat), despite a 4 mg/dL reduction in LDL-C [14]. However, it should be noted that although the women in the low-fat intervention group reduced their percent of energy from total fat by 8.2-percentage points compared to the control group after 6 years (37% vs 28.8%), the 20% goal was not met. Dietary intake data demonstrate that many Americans are not consuming recommended amounts and types of dietary fat (Table 27.2). However, the type of nutrient used to replace fat, and the type of FA consumed markedly affects CVD risk and may be more meaningful than a total dietary target. For instance, certain SFAs and TFAs adversely affect lipid and lipoprotein levels, whereas unsaturated FAs (monounsaturated fatty acid [MUFA] and polyunsaturated fatty acid [PUFA]) are beneficial. Thus, emphasis should be placed on optimizing types of dietary fat and other macronutrients rather than reducing total fat. Based on this evidence, the AHA/ACC 2013 Guideline focuses exclusively on SFA [8].

1 Saturated Fatty Acid

Reducing SFA intake is a common component of dietary guidelines for CVD risk reduction (Table 27.2), and is supported by evidence from epidemiologic, clinical, and animal studies demonstrating that SFA generally increases lipid and lipoprotein levels. Early predictive equations developed by Keys et al. [15] and Hegsted et al. [16] demonstrated that SFA raised TC levels compared to CHO and MUFA (which both had neutral effects), whereas PUFA lowered TC levels. Moreover, SFA was twice as potent in raising TC as PUFA was in lowering TC. Several predictive equations reported that every 1% increase in energy from SFA increased LDL-C levels by approximately 1.28−1.74 mg/dL and HDL-C levels by 0.43−0.50 mg/dL [17−19]. However, individual SFAs may have different effects on lipids and lipoproteins [20]. For instance, stearic acid (18:0) has been shown to have a neutral effect on TC, LDL-C, and HDL-C [21], whereas

TABLE 27.1 Key Recommendations for Foods to Consume More of and Foods to Limit in Order to Achieve a Healthy Dietary Pattern and Reduce CVD Risk[a]

Consume More	Target Servings[b]	Serving Descriptions	Beneficial Components
Vegetables	2½ servings per day[d]	1 cup raw or cooked vegetables or 100% juice; 2 cups leafy salad greens; ½ cup dried vegetable[e]	Fiber, potassium, magnesium, B vitamins, vitamin C, and other phytochemicals
Fruits	1½ servings per day	1 cup of fresh, canned, or frozen fruit; 1 cup 100% fruit juice; ½ cup dried fruit	Fiber, potassium, vitamin C, phytochemicals, and many other nutrients[f]
Whole grains	3 servings per day	1 slice of bread; ½ cup cooked rice, pasta, or cereal; 1 oz. dry pasta or rice; 1 oz. ready-to-eat cereal	Fiber, B vitamins, iron, zinc, phosphorous, riboflavin, vitamin A, phytochemicals, and other micronutrients
Dairy (fat-free or low-fat)	3 servings per day	1 cup milk, yogurt, or fortified soymilk; 1½ oz. natural cheeses such as cheddar cheese; 2 oz. of processed cheese	Calcium, phosphorous, vitamin A, vitamin D (if fortified), fatty acids, protein, magnesium, and other micronutrients
Protein foods	5 servings per day	1 oz. lean meat, poultry, or seafood; 1 egg; ¼ cup cooked beans or tofu; 1 tbsp peanut butter; ½ oz. nuts/seeds	B vitamins, selenium, choline, phosphorous, zinc, copper, vitamin D, and vitamin E
Seafood	8 servings per week	Preferably oily; avoid deep fried or breaded fish	Omega-3 fatty acids, selenium, magnesium, vitamin D, and other nutrients
Meats, poultry, eggs	26 servings per week	Should be consumed in lean forms	Heme iron; meats provide the most zinc; poultry provides the most niacin; eggs provide the most choline
Nuts, seeds, soy products	5 servings per week	Should be unsalted	Nuts/seeds provide the most vitamin E; soy products and legumes are a source of copper, manganese, and iron
Oils	2 servings per day[g]	1 tablespoon of oil; avoid tropical oils with a higher percentage of SFA (e.g., coconut and palm)	Polyunsaturated fats and vitamin E
Consume Less	**Target**	**Strategies to Reduce Intake**	**Beneficial Effects of Reduction**
Solid fats (i.e., SFA and TFA)	Limit intake	Avoid soft margarines with partially hydrogenated vegetable oils, butter, cream, beef tallow, and lard	↓ LDL-C Potentially improves other risk factors
Sodium	Limit to 2300 mg per day	Avoid the "salty six" (i.e., bread and rolls, cured meats, pizza, poultry, soup, and sandwiches)	↓ blood pressure Potentially improves other risk factors
Added sugars	Limit intake	Avoid sweetened beverages, candies, grain-based and other desserts	↓ consumption of empty calories and refined carbohydrates
Alcohol[c]	Up to 2 daily for men; 1 daily for women	Avoid excessive alcohol consumption (1 serving = 5 oz. wine, 12 oz. beer, or 1.5 oz. spirits)	Excessive alcohol intake ↑ blood pressure Moderate alcohol intake ↑ HDL-C and potentially improves other risk factors
Discretionary calories (% total calories)	270 kcal (14%)	If all food choices to meet recommendations are nutrient-dense, a small number of calories (up to the specified limit) can be used for added sugars, refined starches, solid fats, alcohol, or to eat more than the recommended amount of a food group	Maintain energy balance and limit consumption of solid fats, added sugars, and sodium

[a] *Adapted from D. Mozaffarian, L.J. Appel, L. Van Horn, Components of a cardioprotective diet: new insights, Circulation 123 (24) (2011) 2870–2891. [9] and based on the 2015–2020 DGA.*
[b] *Based on a 2000 kcal/day diet. Servings should be adjusted accordingly for higher or lower energy intake.*
[c] *Excessive alcohol consumption increases the risk of alcohol-related accidents, homicides, suicides, and increases chronic disease burden [10]. Thus, alcohol use is not advisable as a population-based strategy to reduce cardiovascular risk and has an overall net adverse effect on population mortality [11]. For adults who already consume alcohol, no more than moderate use should be encouraged, and only when there is no risk of a health condition, drug–alcohol interaction, or adverse effects on work situations.*
[d] *Should include a variety of vegetables from all of the five vegetable subgroups—dark green, red, and orange, legumes (beans and peas), starchy, and other.*
[e] *Should be consumed in a nutrient-dense form, with limited addition of salt, butter, or creamy sauces. When selecting frozen or canned vegetables, choose those lower in sodium.*
[f] *Whole fruit maximizes nutrient intake; fruit juice is lower in fiber and can contribute excess calories.*
[g] *Should replace solid fats rather than being added to the diet.*

TABLE 27.2 Recommended Dietary Fat Intake for Adults (> 19 Years of Age) Compared to Current U.S. Intake

Type of Fat	Recommended Intake (% of Daily Calories)[a]				U.S. Adult Intake (% of Daily Calories)[a]
	2015–2020 DGA	AHA/ACC 2013	FAO/WHO 2005	AND 2007	
Total fat	20–35	–[b]	20–35	20–35	33–34
SFA	<10	5–6	10	Goal of <7; 10% maximum	11
TFA	–[c]	–[c]	<1	<1	<1
MUFA	–[d]	–	–[e]	15–20	12–13
PUFA	–[d]	–	6–11	–	7–8
Omega-3	–	–	0.5–2	0.5–2	<1
Omega-6	–	–	2.5–9	5–10	7

[a] *From NHANES What We Eat in America 2011–12.*
[b] *Not specified in 2013 publication. Previous AHA Diet and Lifestyle Guideline specified 25–35%.*
[c] *No amount specified, but intake should be kept as low as possible.*
[d] *No amount specified, but recommend replacing foods high in SFA with foods high in unsaturated fats.*
[e] *No amount specified, but can be up to 15–20% according to total fat intake.*
ACC, American College of Cardiology; AND, Academy of Nutrition and Dietetics; AHA, American Heart Association; DGA, Dietary Guidelines for Americans; FAO, Food and Agriculture Organization of the United Nations; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TFA, *trans* fatty acid; WHO, World Health Organization.

myristic acid (14:0) is more hypercholesterolemic than lauric acid (12:0) and palmitic acid (16:0) [22]. Despite these differential effects, recommendations targeting individual FAs are not practical because people generally consume SFA as a blend in food sources. SFA intake may also adversely affect other CVD risk factors, such as inflammation [23,24] and vascular function as assessed by flow-mediated dilation [25,26], but clinical evidence is limited and inconsistent [25,27].

Despite many decades of dietary guidelines advising reduced SFA intake, the association between dietary SFA and CVD risk remains controversial and highly debated. Although SFA intake has been associated with greater CVD incidence in some epidemiological studies [28–30], many systematic reviews and meta-analyses have found no association for CVD risk [31–34]. For instance, in a meta-analysis of prospective cohort studies evaluating FAs and CHD risk, Skeaff and Miller found no association between SFA intake and the risk of CHD death or events [31]. Table 27.3 shows the estimated relative risk associated with each FA in this analysis. Subsequent meta-analyses have similarly questioned the association between dietary SFA intake and increased CVD risk [32–34]. However, it should be noted that a major limitation of these studies is that they failed to evaluate nutrient substitutions for SFA (discussed further below).

Importantly, decreasing SFA intake is typically accompanied by an increase in another macronutrient and this has a substantial effect on lipid/lipoprotein responses, and thereby influences overall CVD risk. For instance, substituting SFA with MUFA, PUFA, or CHO consistently lowers both LDL-C and HDL-C [8]. However, the replacement of SFA with CHO or MUFA raises TG, whereas replacement with PUFA lowers TG [8]. Thus, the lack of association between SFA and CVD risk in some meta-analyses may be due to insufficient consideration of the type of macronutrient used to replace SFA. For instance, in a pooled analysis of 11 cohort studies from both the United States and Europe [35], replacing 5% of energy intake from SFA with PUFA reduced the risk of CHD death (hazard ratio, HR: 0.74; 95% CI: 0.61–0.89) and CHD events (HR: 0.87; 95% CI: 0.77–0.97). In contrast, substitution with MUFA was not associated with CHD, and substitution with CHO was associated with a modest increased risk of CVD events (HR: 1.07; 95% CI: 1.01–1.14), but not CVD death. However, it should be noted that there was no discrimination between CHO sources in this study [35], which can significantly affect CHD risk (discussed further in following sections). Furthermore, in a 2015 analysis of 84,628 women in the Nurses' Health Study and 42,908 men in the Health Professionals Follow-up Study, isocaloric (5%) substitution of SFA with PUFA, MUFA, and CHO from whole grains was associated with a 25%, 15%, and 9% lower risk of CHD, respectively (Fig. 27.1) [36]. It has also been estimated that each 5% energy increase in

TABLE 27.3 Estimated Relative Risk of CHD Death and CHD Events for Individual Fatty Acids in Prospective Cohort Studies[a]

Type of Fat	Relative Risk (95% CI)				Mean Intake in Low and High Categories Among Cohorts (% Total Energy)	
	CHD Death	*p* Value	CHD Events	*p* Value	Low Intake	High Intake
Total fat	0.94 (0.74–1.18)	0.583	0.93 (0.84–1.03)	0.177	23–30	38–47
TFA	1.32 (1.08–1.61)	0.006	1.25 (1.07–1.46)	0.007	0.8–2.4	1.6–6.4
SFA	1.14 (0.82–1.60)	0.431	0.93 (0.83–1.05)	0.269	7–11	14–18
MUFA	0.85 (0.60–1.20)	0.356	0.87 (0.74–1.03)	0.110	9–11	16–20
PUFA	1.25 (1.06–1.47)	0.009	0.97 (0.74–1.27)	0.825	3–4	6–10
n-3 LC-PUFA	0.82 (0.71–0.94)	0.006	0.87 (0.71–1.10)	0.066	0–0.3 g/day	0.37–2.5 g/day

[a]*Relative risks correspond to comparisons between the highest and lowest intakes.*
CHD, coronary heart disease; LC, long chain; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TFA, *trans* fatty acid.
Source: Adapted from C.M. Skeaff, J. Miller, Dietary fat and coronary heart disease: summary of evidence from prospective cohort and randomised controlled trials, Ann. Nutr. Metab. 55 (1–3) (2009) 173–201.

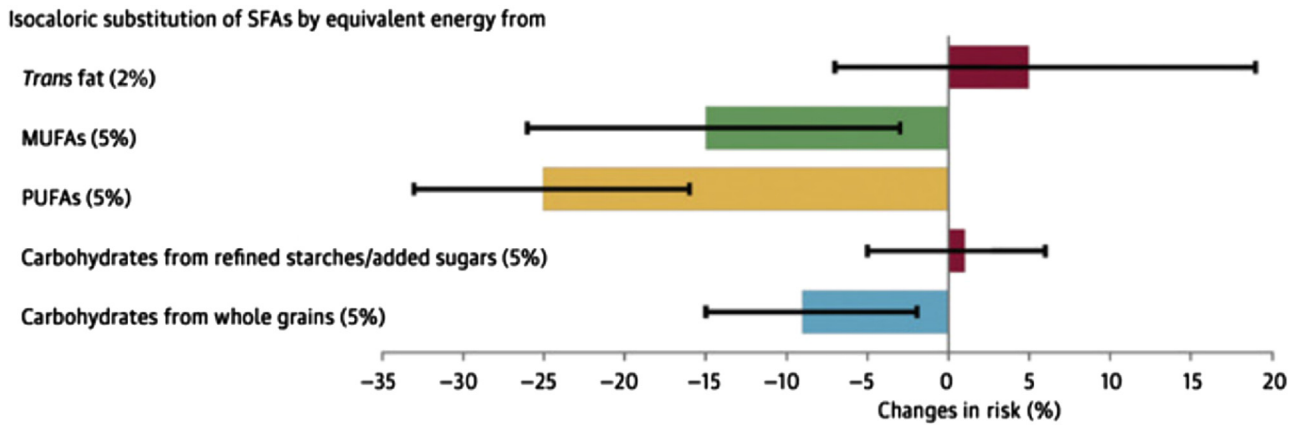

FIGURE 27.1 Estimated percent change in the risk of CHD associated with isocaloric substitution of saturated fat. *Adapted from Y. Li, et al., Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study, J. Am. Coll. Cardiol. 66 (14) (2015) 1538–1548 [36].*

PUFA as a replacement for SFA decreases CVD events by 10% [37].

More research is needed to clarify discrepancies about the association between SFA intake and CVD risk among epidemiologic studies. Particular focus on evaluating the specific nutrients (i.e., types of fat and CHO) replacing dietary SFA intake would help to more accurately determine effects on CVD risk. Although the macronutrient substitution for SFA was not evaluated in the previously mentioned meta-analyses that found no association between SFA intake and CVD risk [31–34], these analyses may also be limited by inconsistent and/or imprecise dietary assessment methods, the potential for regression dilution bias, underestimation of an association due to variability, and/or measurement error. There is also growing evidence that the food source of SFA may influence CVD risk [27,38,39]. Furthermore, there is still debate about whether MUFA, PUFA, or complex CHO are the optimal replacement for SFA.

2 Trans *Fatty Acid*

TFAs are classified as unsaturated FAs that have at least one nonconjugated double bond with *trans*

stereochemistry. Dietary TFA is primarily a result of industrial production (e.g., partially hydrogenated oils), although some *trans* isomers are produced by ruminant animals. The adverse effects of TFA on cardiovascular health have been demonstrated in both clinical and epidemiologic trials. TFA intake consistently increases TC, LDL-C, and the TC/HDL-C ratio, and decreases HDL-C [40,41]. Moreover, there is substantial evidence that TFA increases the risk of CVD death and CVD events more than any other type of FA [31,42,43]. In 2003, based on widespread scientific agreement regarding the adverse effect of TFA consumption on CHD risk, the Food and Drug Administration issued a final rule requiring the declaration of the amount of TFA present in foods and dietary supplements [44]. Labeling requirements and growing public awareness have also led to efforts to reduce TFA content in restaurant foods and reformulate food products to eliminate or substantially reduce TFAs.

The naturally occurring TFA in ruminant meat and dairy products is predominantly composed of vaccenic acid (C18:1 *trans*-11) and the isomer of conjugated linoleic acid (CLA; C18:2 *cis*-9, *trans*-11). It has been proposed that the structure of bonds in CLA may confer different physiological effects compared to industrial TFA, but this remains unclear in both epidemiological and clinical studies. In two quantitative reviews of randomized controlled trials (RCTs), Brouwer et al. [45,46] found that gram for gram, all types of TFA have largely the same unfavorable effect on LDL-C, the LDL-C/HDL-C ratio, and the TC/HDL-C ratio. However, it should be noted that the amount of ruminant TFA provided in the majority of clinical studies greatly exceeds the amount typically consumed (<2%) [47]. The amount of ruminant TFA in the diet is also minimal relative to industrial TFA, and it is much more likely for industrially produced TFA to be consumed at amounts that adversely affect CVD risk factors. Maintaining a low-SFA intake is likely to ensure that ruminant TFA is minimal [47]. Therefore, while it is possible that industrial and ruminant TFA have different metabolic effects, there is currently no conclusive evidence to justify excluding ruminant TFA from the recommendation to reduce total TFA consumption as much as possible [7,8,47].

3 Monounsaturated Fatty Acid

MUFAs are classified as FAs containing a single double bond. The most predominant MUFA in the diet is oleic acid (18:1*n*-9), which is a primary component of vegetable oils (e.g., olive oil) and nuts. There is a large body of evidence demonstrating that the replacement of SFA with MUFA improves multiple CVD risk factors [48–50]. For instance, using MUFA to isocalorically replace SFA reduces TC, LDL-C, and the TC/HDL-C ratio [8,51,52]. As a SFA replacement, MUFA also tends to preserve HDL-C to a greater extent than PUFA [49]. Furthermore, when CHO are isocalorically replaced by MUFA, HDL-C is increased while LDL-C and TG are lowered [8,17,52]. MUFA intake may also lower systolic and diastolic BP [50] and improve insulin sensitivity and the glycemic response [49]. Some have challenged the cardioprotective effects of MUFA based on results from animal studies [53,54], but no detrimental effects have been reported in human studies [55].

Many epidemiological studies support cardioprotective benefits of MUFA for CVD risk [29,56,57], but not all studies have been consistent [35,58]. In a systematic review of prospective cohort studies, Mente et al. [56] reported an inverse association between MUFA and CHD risk (risk ratio (RR): 0.80; 95% CI: 0.67–0.93). Moreover, a 5% isocaloric replacement of MUFA for SFA has also been associated with lower CVD risk [29,57]. In contrast, the pooled analysis of prospective cohort studies by Jakobsen et al. [35] found that substituting MUFA in place of SFA was associated with an increased risk of CHD events (HR: 1.19; 95% CI: 1.00–1.42). However, these positive correlations may be due to confounding with SFA intake as food sources of MUFA in the United States tend to also be high in SFA (e.g., meat and dairy) [35,58].

Dietary guidelines for reducing CVD risk now increasingly recommend MUFA (and PUFA) as a replacement for SFA [7]. Higher-MUFA intake is also a key component of the Mediterranean-style dietary pattern due to the emphasis on olive oil over other fats. In the United States, MUFA intakes are typically ~13–14% of energy, whereas they comprise ~16–29% of energy in Mediterranean-style diets—largely at the expense of SFA [59]. The particular food source of MUFA is also an important consideration when considering CVD risk. For instance, the predominant sources of oleic acid in the typical American diet are grain-based desserts, meat, and poultry [60]. It is unlikely that greater MUFA intake would result in cardioprotective effects in this context. If MUFA intake is increased, it should be obtained from MUFA-rich vegetable oils, such as olive or canola oil, and/or nuts. Current clinical evidence indicates that MUFA is a suitable substitution for SFA, TFA, and/or CHO; however, there are considerably fewer studies that have investigated the effects of MUFA on CVD risk compared to the number on PUFA and no RCTs have been conducted to evaluate the effect of MUFA on CVD morbidity and/or mortality.

4 Polyunsaturated Fatty Acid

There are two major classes of PUFA, and they are defined by the position of the first double bond relative to the methyl terminus: omega-6 (*n*-6) and omega-3 (*n*-3) FA.

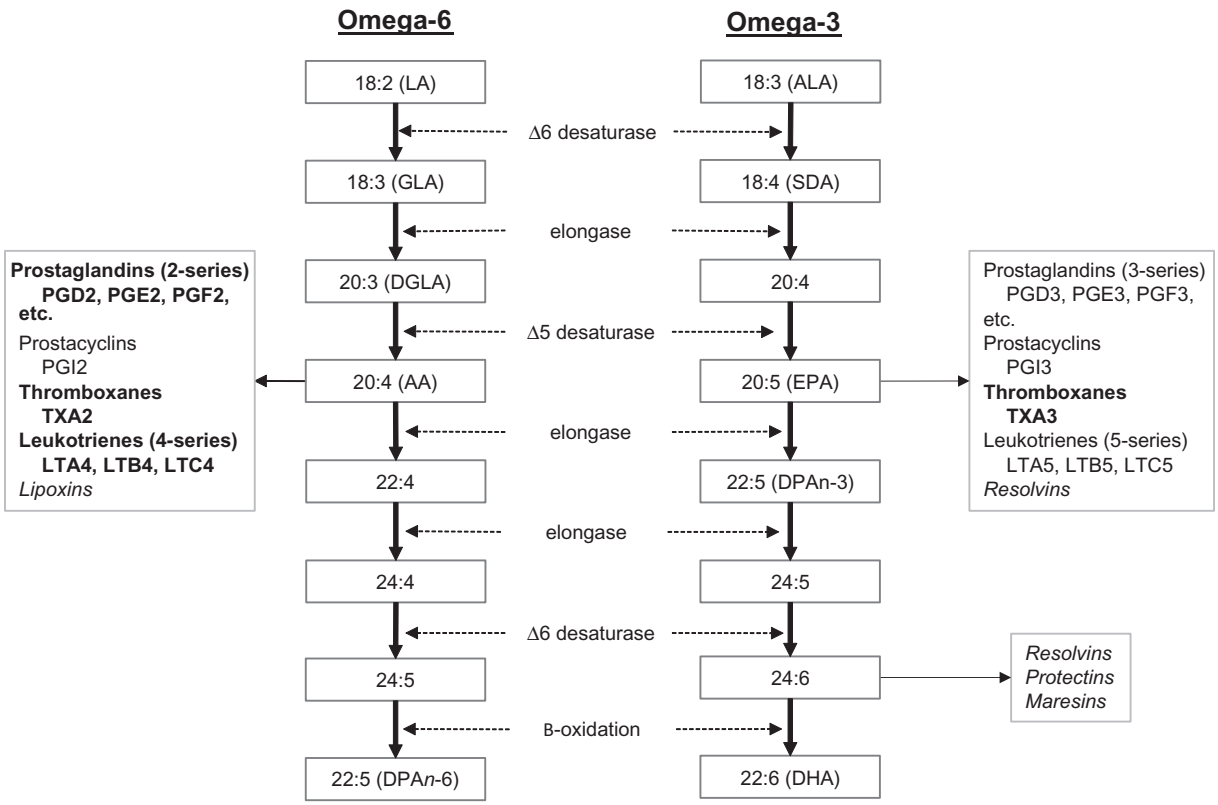

FIGURE 27.2 Biochemical pathway for the interconversion of *n*-6 and *n*-3 FAs. AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; SDA, stearidonic acid. Bold text = proinflammatory; normal text = less inflammatory; and *italicized* text = proresolving.

Linoleic acid (LA; C18:2*n*-6) and α-linolenic acid (ALA; C18:3*n*-3) are the essential *n*-6 and *n*-3 FAs. In the body, LA and ALA can undergo a series of elongations and desaturations to yield arachidonic acid (AA; C20:4*n*-6), and eicosapentaenoic acid (EPA; C20:5*n*-3) and docosahexaenoic acid (DHA; C22:6*n*-3), respectively (Fig. 27.2). However, the amount of ALA converted to EPA and *especially* DHA is very low [61] and these FAs are obtained primarily from fatty fish in the diet [62,63].

Dietary PUFAs have many physiological functions. EPA and AA are metabolized into a variety of hormone-like compounds called eicosanoids, which include prostaglandins, thromboxanes, leukotrienes, and lipoxins. These compounds play a key role in inflammatory and thrombotic pathways. AA, EPA, and DHA are also the precursors for lipoxins [64,65], resolvins, protectins, and maresins [66,67] that actively assist in the resolution of inflammation. Concerns have been raised that PUFA may be more susceptible to oxidation, and thus may have adverse health effects; however, this is unlikely within the context of a healthy dietary pattern.

Many longer term clinical studies have shown that increased PUFA intakes improve lipids/lipoproteins and lower CVD risk [29,31,35–37,68]. Based on predictive equations [16,28], a 1% increase in energy from PUFA translates to a 0.9 mg/dL decrease in TC. Replacement of CHO with PUFA also increases HDL-C [52], albeit to a lesser extent than MUFA [8]. Early controlled trials verified these hypocholesterolemic effects of high PUFA diets and also found an associated reduction in the incidence of CVD (16–34%) [69–71]. A dose–response relationship between PUFA intake and CVD risk was also reported in the Nurses' Health Study, with the highest quintile of intake (6.4% of calories) conferring an approximately 30% reduction in risk [29]. However, there remains much debate over the relative benefits of *n*-6 versus *n*-3 PUFA, as well as the effects of individual PUFAs (i.e., EPA, DHA, and ALA).

a Eicosapentaenoic Acid and Docosahexaenoic Acid

Interest in EPA and DHA was first generated by the seminal studies of Dyerberg et al. [72], in which the low incidence of CHD among Greenland Eskimos was attributed in part to a high intake of marine oils rich in EPA and DHA. Numerous epidemiological studies and RCTs have since confirmed that these FAs reduce CVD risk and confer cardioprotective effects via multiple mechanisms of

action, and have been reviewed previously in detail [73–75]. Of particular note are the key large-scale intervention studies that first demonstrated the beneficial effects of *n*-3 FA for primary and secondary prevention, including The Diet and Reinfarction Trial (DART), Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione, GISSI-Heart Failure, and Japan EPA Lipid Intervention Study (JELIS). In DART—the first RCT to investigate the effects of *n*-3 FA on secondary prevention of MI—men advised to consume oily fish 2–3 times per week experienced a 29% reduction in all-cause mortality [76]. Further evidence for the use of EPA and DHA in secondary prevention of CVD was provided by GISSI-P and GISSI-HF. In GISSI-P, *n*-3 supplementation (1 g/day) resulted in a 15% reduction in the combined primary endpoint of death and nonfatal cardiovascular events [77], and in GISSI-HF the intervention (1 g/day) reduced all-cause mortality by 9% [78]. JELIS was the first large-scale, prospective RCT to evaluate *n*-3 supplementation for primary prevention, as well as the combined effect of statin treatment and EPA supplementation. In this study, supplementation with 1.8 g/day EPA and statin treatment reduced major coronary events by 19% compared to the control group (statin only) [79]. This finding was also particularly notable given the much higher background *n*-3 FA intake of Japanese individuals compared to Americans. These studies provide much of the basis for recommendations to increase EPA and DHA to reduce CVD risk.

Unfortunately, many of the more recent clinical studies have failed to replicate the findings from earlier trials [80–82]. For instance, in the ALPHA-OMEGA trial, there was no significant difference in cardiovascular events among those who received 400 mg/day EPA + DHA compared to the control group [80]. Many have attributed these null findings to several key differences in study design [73,83,84]. For instance, in the more recent trials, a large proportion of participants were concurrently using blood pressure and/or lipid-lowering medications that may have overwhelmed any potential effects of *n*-3 FAs [83]. Furthermore, many of the studies were underpowered to detect effects on CHD mortality and/or had lower than expected event rates [85]. Other potential explanations for the absence of effects include: insufficient dosing, failure to assess the *n*-3 status of participants prior to and during treatment, and insufficient treatment duration [73,83,85]. Due to these concerns, it is unlikely that these results from more recent studies will drastically alter dietary recommendations for *n*-3 intake. However, future RCTs of *n*-3 FAs should be designed with these factors in mind, and are needed to clarify these discrepancies.

Intermediate risk factors for CVD can also be improved by greater EPA + DHA intake. Fish oil has a marked hypotriglyceridemic effect in individuals with normal or elevated TG levels (> 150 mg/dL). In 21 studies that examined the effects of fish or fish oil (0.1–5.4 g/day EPA + DHA) on lipids and lipoproteins, EPA + DHA dose-dependently reduced TG, with a net effect of −27 mg/dL [86]. The TG-lowering effects of fish oil are also stronger in individuals with elevated TG [87]. Although there is substantial preclinical evidence of the antiinflammatory potential of EPA and DHA [88], this has not translated to consistent results on inflammatory outcomes in clinical intervention studies [89,90]. EPA and DHA can also lower resting heart rate and BP, and may improve endothelial function [85]; however, the effect of these changes on clinical events (i.e., CHD death) tends to have varying time courses and depend on the dose of EPA + DHA (Fig. 27.3) [75]. For instance, at intakes of <750 mg/day, antiarrhythmic effects predominate and can reduce clinical events within weeks. This has

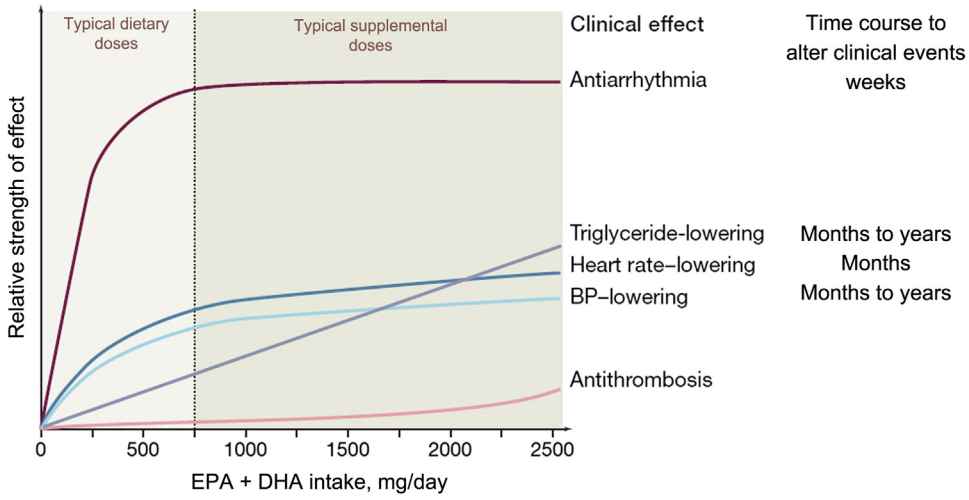

FIGURE 27.3 Schema of potential dose responses and time courses for altering clinical events due to the physiologic effects of EPA + DHA intake. Source: *D. Mozaffarian, E.B. Rimm, Fish intake, contaminants, and human health: evaluating the risks and the benefits, JAMA 296 (15) (2006) 1885–1899.*

important clinical implications as arrhythmias are the primary cause of sudden cardiac death. Conversely, a reduction in TGs is hypothesized to require a longer period of time to reduce clinical events (i.e., months or years) and to have a stronger effect at higher doses of EPA + DHA [75]. Ongoing clinical studies are examining whether TG-lowering doses of *n*-3 reduce CVD mortality (Reduction of Cardiovascular Events with EPA—Intervention Trial [REDUCE-IT], NCT01492361 and STatin Residual Risk Reduction with EpaNova in HiGh Cardiovascular Risk PatienTs With Hypertriglyceridemia [STRENGTH], NCT02104817).

Concerns have been raised regarding potential increases in LDL-C with greater EPA + DHA intake. In an evaluation of 15 RCTs, the Agency for Healthcare Research and Quality found that marine *n*-3 FA consumption (ranging from 45 to 5400 mg/day of EPA and/or DHA) resulted in a net LDL-C increase of 10 mg/dL [91]. This may be due in part to an increase in LDL particle size [92,93] and is thereby less atherogenic, although some have found no change in LDL particle size following *n*-3 supplementation [94]. It has also been proposed that the change in size depends on an individual's baseline LDL particle size. Those who have a greater amount of small dense LDL particles—termed a pattern B phenotype—tend to respond to fish oil supplementation with an increase in LDL particle size, whereas others do not [94]. A prescription dose of EPA and DHA (3.4 g/day EPA + DHA) can be combined with statin therapy to attenuate the increase in LDL-C [95]. Physician oversight is recommended when doses exceed 2 g/day EPA + DHA for TG reduction [96].

Another area of concern when recommending increased fish intake pertains to the issue of environmental contaminants such as methylmercury. Most fish contain trace amounts of methylmercury from natural sources, but this can be exacerbated by environmental contamination from industrial practices. Larger, older, and higher trophic-level species generally have higher methylmercury content because methylmercury is readily absorbed by tissues and tends to bioaccumulate [97]. However, the majority of fatty fish that are regularly consumed have relatively low methylmercury concentrations (Fig. 27.4) [75]. Consumption of certain fish high in methylmercury (e.g., shark, tilefish, king mackerel, and swordfish) should be avoided by at-risk population groups (i.e., women who are pregnant, could become pregnant, or are lactating, and infants and young children) [99]. State and local advisories for safe fish consumption should also be followed. Although increased methylmercury intake may modestly decrease the benefits of fish intake [75,99], the health benefits of oily fish consumption far outweigh the potential risks [75,100,101]. Thus, emphasis should be placed on consuming species that are high in *n*-3 FA and also low in methylmercury.

Fish oil supplements can also be used to increase EPA and DHA intake. Fish oil supplements can be cost-effective, convenient, and typically contain negligible quantities of environmental contaminants [102]. Some individuals may prefer supplements due to personal preferences, such as ethical or environmental concerns, disliking the taste of fish, unfamiliarity with seafood preparation and cooking methods, cost, food allergies, and a perceived risk of pollutants. Others may require a supplement to achieve higher recommended LC *n*-3 PUFA intakes that are not attainable by dietary means alone (i.e., those with established CHD or elevated TGs).

A pooled analysis of prospective studies and randomized clinical trials found that modest consumption of fish and/or fish oil (e.g., 1−2 servings/week providing ~250 mg/day of EPA + DHA) reduced CHD death (~35%), sudden death from arrhythmia (~50%), and ischemic stroke (~30%) when compared to little or no intake [75]. Modest benefits were also reported for nonfatal MI, delayed progression of atherosclerosis, recurrent ventricular tachyarrhythmias, and postangioplasty restenosis. However, a threshold of effect has been proposed because higher intakes (e.g., >900 mg/day) do not elicit a greater decrease in risk [75,103]. Based on this evidence, numerous expert authorities have issued recommendations for amounts of fish and/or LC *n*-3 PUFA intake to promote health and reduce CVD risk, with the general consensus specifying intake of 250−500 mg of EPA + DHA per day or 2−3 servings of oily fish per week. For instance, in addition to their dietary pattern recommendations, the AHA has also issued specific guidelines for EPA and DHA intake (Table 27.4) [96]. The 500 mg/day of EPA + DHA recommended for the primary prevention of CHD can be achieved by consuming two servings (4 oz. each) of fatty fish per week. Frequent consumption of oily fish is also a characteristic feature of Mediterranean-style dietary patterns [105,106].

b Alpha-Linolenic Acid

There remains debate as to whether increased dietary ALA confers the same cardioprotective effects as EPA and DHA [107−110]. In a pooled meta-analysis of 27 prospective and retrospective studies comprising over 250,000 individuals, higher ALA exposure was associated with a moderate reduction in CVD risk (RR: 0.86; 95% CI: 0.77−0.97) [110]. However, when analyses were performed separately based on self-reported dietary intake or biomarker data, only self-reported dietary intake of ALA remained significantly associated with reduced CVD risk. With regard to ALA supplementation in RCTs, the Lyon Diet Heart Study is the largest clinical trial to examine

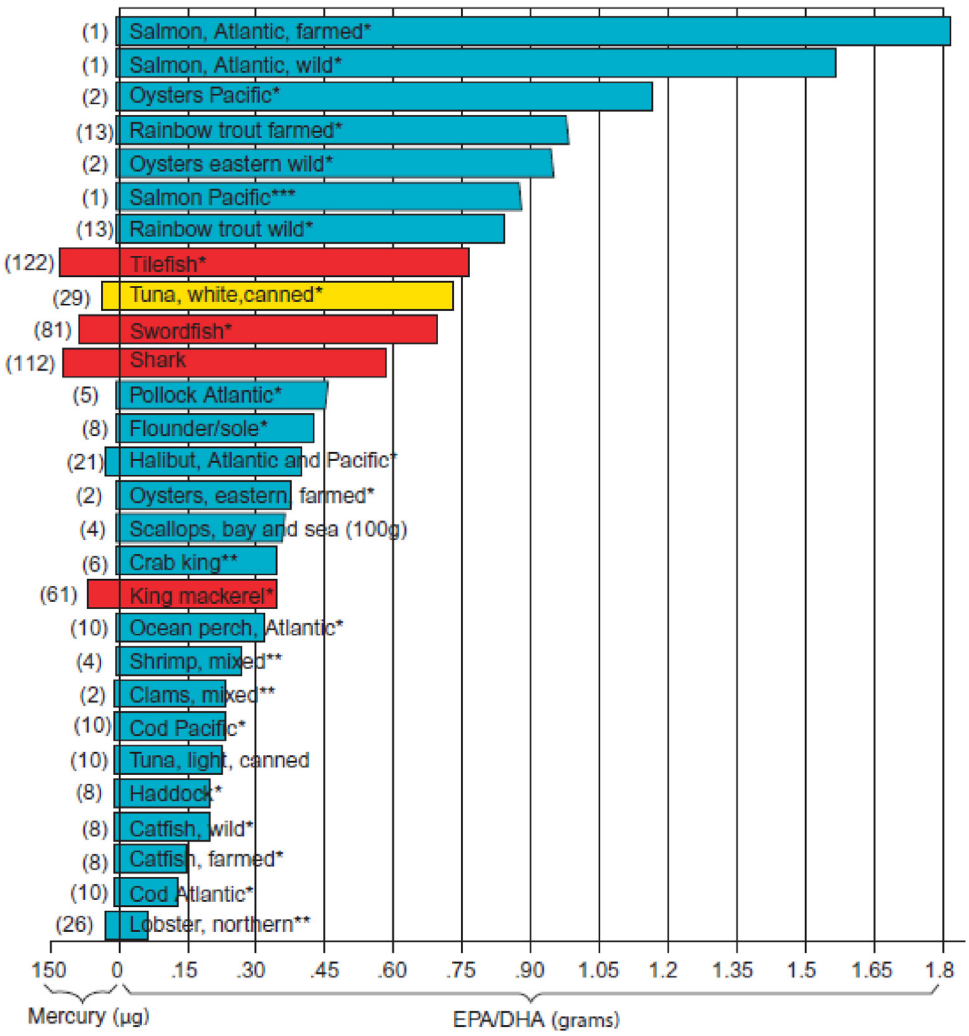

FIGURE 27.4 Estimated EPA + DHA and methylmercury content of different seafood species (per 3 oz. portion). *Cooked, dry heat; **cooked, moist heat; ***the EPA + DHA content in Pacific salmon is a composite from chum, Coho, and sockeye species. Species indicated in black should be avoided by at-risk population groups (i.e., women who are pregnant, could become pregnant, or are lactating, and infants and young children) due to methylmercury concentrations that exceed recommended safe intakes. Gray indicates that consumption should be limited to 6 oz. per week for at-risk population groups. Source: *M. C. Nesheim, A.L. Yaktine, Seafood Choices: Balancing Benefits and Risks, National Academies Press, Washington, DC, 2007.*

TABLE 27.4 Summary of AHA Recommendations for Omega-3 FA Intake

Population	Recommendation
Patients without documented CHD	Eat a variety of (preferably fatty) fish at least twice a week. Include oils and foods rich in ALA (flaxseed, canola, and soybean oils; flaxseed and walnuts).
Patients with documented CHD	Consume ≈1 g of EPA + DHA per day, preferably from fatty fish. EPA + DHA in capsule form could be considered in consultation with the physician.
Patients who need to lower TGs	2–4 g of EPA + DHA per day provided as capsules under a physician's care.

Source: Kris-Etherton, P.M., W.S. Harris, and L.J. Appel, Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation, 2002. 106 (21): 2747–2757

the effects of a high-ALA diet high on CVD. The advice to follow a Mediterranean-style diet high in ALA resulted in a 70% reduction in cardiac death and nonfatal MI and a 50% reduction in all coronary events, despite no improvement in lipids and lipoproteins [111,112]. The authors attributed these benefits largely to the 68% increase in ALA intake (~1.7 g/day) in the experimental group [111]. However, the AHA Science Advisory suggested that other differences in the intervention diet (e.g., increased bread, root vegetables and green vegetables,

and fish; fruit at least once daily; more poultry in place of red meat; and high-ALA margarine in place of butter and cream) likely also played a role [113]. Other systematic reviews have concluded that short-term trials (6–12 weeks) of 1.2–3.6 g/day ALA intake generally show inconsistent effects on lipids/lipoproteins, markers of inflammation and oxidative stress, and glucose metabolism [109]. Thus, many questions remain regarding the cardiovascular benefits of ALA. Additional long-term RCTs are needed to examine the unique effects of ALA on CVD outcomes and thereby inform recommendations for optimal intake.

c Omega-6 (*n*-6) Versus Omega-3 (*n*-3) PUFA

Although *n*-6 FA intake has previously been shown to reduce the risk of CVD [37,114], some have expressed concern that increased *n*-6 FA consumption will inhibit *n*-3 FA metabolism because these FAs share common enzymatic pathways (Fig. 27.2). This inhibition of *n*-3 FA metabolism is hypothesized to occur at two biological steps. First of all, an increase in LA may interfere with the conversion of ALA to EPA because LA utilizes the same desaturase and elongase enzymes to form AA. Secondly, AA, EPA, and DHA are metabolized into eicosanoids and other oxygenated metabolites by the same enzymes; thus, it has been proposed that greater dietary intake of *n*-6 FAs (and thus greater *n*-6 FA concentrations in cell membranes) would result in a greater proportion of proinflammatory lipid mediators derived from AA.

Therefore, rather than increasing *n*-3 FA intake, some have instead advocated for targeting a lower *n*-6 to *n*-3 ratio by reducing the intake of LA [115–119]. This can be achieved by substituting high *n*-6 foods (e.g., vegetable oils, nuts, and seeds) with MUFA and using olive and canola oils for cooking and dressings. However, there is a paucity of research directly comparing increased dietary *n*-6 FA intake to a higher-MUFA diet. Much of the clinical evidence supporting the substitution of MUFA for LA is based on comparisons of Mediterranean-style and Western dietary patterns. In these comparisons, the high *n*-6 vegetable oils that predominate in a Western dietary pattern are often used for frying and the preparation of foods that are relatively low in fiber and other plant-based bioactives (e.g., phytosterols and phytochemicals). In contrast, the high-MUFA olive oil that is characteristic of a Mediterranean-style dietary pattern typically accompanies grains and vegetables, and provides additional antioxidants that are not present in refined vegetable oils. Furthermore, although nuts are high in LA they are also rich in many other bioactive compounds. Thus, these types of comparisons are not directly comparing dietary LA and oleic acid, and cannot account for the complexity of these factors.

It is likely that the ratio of *n*-6 to *n*-3 FA is less important than the total amount of *n*-3 FA intake [120], and its value as a clinically relevant risk factor has been challenged [121,122]. Concerns about the inflammatory effects of *n*-6 FA intake are based primarily on cell-based studies. In humans, LA intake has not been associated with elevated concentrations of inflammatory markers [123]. Moreover, this approach also fails to recognize that LA is an essential FA and that AA also serves as the substrate for proresolving lipoxins [64,65]. Based on evidence to date, dietary advice on PUFAs should encourage increased consumption of ALA, EPA, and DHA, without instruction to limit the intake of plant sources of *n*-6 FAs, such as nuts and seeds.

B Dietary CHO

The characteristics and/or type of dietary CHO consumed are also important considerations. Each of the evidence-based dietary patterns discussed in this chapter recommends decreasing the consumption of foods containing refined CHO (particularly those that are also high in SFA and added sugars) and replacing them with nutrient-dense foods. Fruits, vegetables, legumes, and whole grains are all nutrient-dense high-CHO foods. It is likely that many non-CHO components of these foods contribute to their health benefits (e.g., vitamins, minerals, phytochemicals, etc.), and it is difficult to attribute specific effects to a particular compound. The potential effects of each of these components are beyond the scope of this chapter; thus, the following sections will focus on key areas of research related specifically to CHO and CVD risk.

1 Dietary Fiber

Dietary fiber can be broadly defined as nondigestible CHO polymers of plant origin, but this encompasses a wide range of compounds with differing effects on gastrointestinal and systemic physiological processes. Traditionally, dietary fiber has been categorized as soluble or insoluble based on whether it dissolves in water to become a gelatinous substance. However, there remains much debate regarding the optimal definition for classifying different types of dietary fiber and the Institute of Medicine (IOM) has recommended that “soluble” and “insoluble” be replaced by terms that better account for physiochemical properties, such as viscosity and fermentability. These properties are essential to the mechanism by which these compounds modify CVD risk factors, but both soluble and insoluble fiber are associated with reduced CVD risk [124]. Although an optimal amount of dietary fiber is not specified, evidence-based dietary patterns typically emphasize fiber-rich foods (e.g., whole

grains, vegetables, fruits, legumes, and nuts). Each of these foods are key sources of dietary fiber but it is possible that dietary fiber from particular foods may be more strongly associated with decreased CVD risk [124,125] due to other nonfiber components (e.g., vitamins, minerals, phytochemicals, etc.).

The relationship between dietary fiber intake and CVD risk has been extensively studied and reviewed by others [124,126,127]. In brief, there is substantial epidemiological evidence for an inverse association between dietary fiber intake and CVD risk [124]. Of particular note is a pooled analysis of 10 prospective cohort studies from the United States and Europe, in which each 10 g/day increment of dietary fiber yielded a 27% reduction in the risk of coronary mortality and a 14% reduction in all coronary events [128]. Subsequent systematic reviews of cohort studies have reported similar inverse associations [56,125]. The cholesterol-lowering effects of dietary fiber are also well-documented [127], and are attributed primarily to increased bile acid excretion and reduced cholesterol and fat absorption. There is also strong evidence that regular consumption of dietary fiber may improve blood pressure, body weight regulation, and glucose metabolism [124]. Prolonged satiety and delayed gastric emptying likely plays a role in these effects, but additional research is needed to clarify the biological mechanisms responsible for the cardioprotective effects of dietary fiber.

a Gut Microbiome

The human colon is colonized by a dense population of microorganisms—the majority of which are bacteria that have coevolved in a symbiotic relationship with the body's own cells. There is growing evidence that the composition and/or function of the gut microbiota is strongly linked to the development of multiple diseases [129,130], including CVD [131]. Diet is a major determinant of the composition of the gut microbiota as nondigestible CHO (e.g., resistant starch, nonstarch polysaccharides, and oligosaccharides) serve as the primary substrate for microbial metabolism [132,133]. Diets that are higher in fiber have been associated with a greater abundance of bacterial species that are considered beneficial, such as *Bifidobacterium* [132,133]. Furthermore, the short-chain FAs produced from microbial fermentation of CHO (i.e., acetate, propionate, and butyrate) can directly alter human health [134]. For instance, butyrate contributes to colonic health and has been shown to have anti-inflammatory and anticarcinogenic potential, whereas propionate can inhibit cholesterol synthesis [135]. Increased consumption of resistant starch has been associated with greater abundance of microbial species known to produce short-chain FAs [132,136], whereas diets with less CHO content have been shown to result in diminished production of butyrate and other short-chain FAs [137–139]. Therefore, this is a promising new area of research for reducing CVD risk and provides further support for the whole-food-based approach of the dietary patterns discussed in this chapter.

2 Glycemic Index and Glycemic Load

CHO can also be classified according to their effects on the glycemic response, which can be quantified as the glycemic index (GI) and the glycemic load (GL). Low GI foods (e.g., mature beans and green vegetables) elicit less of a glycemic response than high GI foods (e.g., potatoes and ready-to-eat cereals). Foods that are high in soluble fiber tend to have a low GI, but the GI can be altered by food preparation methods and the consumption of other foods in the context of a mixed meal. The GL incorporates both the type and amount of CHO, and estimates the effect of consuming a particular food based on its GI and the amount of CHO ingested.

However, there remains some debate as to the utility of the GI and the GL for reducing CVD risk. Overall, evidence from epidemiological studies indicates that CVD risk is increased by consuming high GI/GL diets [140] and low-GI diets have been associated with reduced risk of type 2 diabetes [141]. However, recent meta-analyses of cohort studies have suggested that the association between high GI/GL diets and increased CVD risk is stronger in women, and not present in men [142–145]. The association between low GI/GL diets and reduced risk may be due to beneficial effects on CVD risk factors. For instance, a low-GI diet has been recommended for weight loss [146] and as a strategy for diabetes management based on improvements in glycemic control [147]. A meta-analysis of RCTs comparing low-GI diets to high-GI diets over at least 4 weeks also found that lowGI diets significantly reduced TC (−5 mg/dL) and LDL-C (−6.2 mg/dL) without altering HDL-C or TG [148]. Conversely, a meta-analysis of the long-term (≥ 6 months) effects of low GI/GL diets found a significant reduction in C-reactive protein (CRP), fasting insulin, and fat-free mass but no change in blood lipids [149]. Results from controlled feeding studies of low-GI diets have also been inconsistent [150] and a low-GI diet has not been shown to improve insulin sensitivity, lipids/lipoproteins, or systolic BP compared to a healthful Dietary Approaches to Stop Hypertension (DASH)-type diet [151]. Based on these contradictory findings, there is a lack of consensus among the expert authorities regarding whether GI/GL should be emphasized in dietary recommendations [152]. Additional research is needed to clarify which

CVD risk factors are most effectively addressed by reducing the GI/GL of the diet.

3 High- and Low-Carbohydrate Diets

Replacing SFA with CHO is one strategy for reducing LDL-C; however, the resulting high-CHO, low-fat diet also tends to decrease HDL-C and increase TG [8]. This can be particularly problematic for people with insulin resistance and accompanying dyslipidemia characterized by low HDL-C and elevated TG [153]. As previously discussed, the effect of increasing CHO intake is likely dependent on the type of CHO (and the type of fat being replaced). Recommendations to replace fat with CHO should emphasize nutrient-dense vegetables, fruits, legumes, and whole grains in place of refined CHO, SFA, and *trans* fat [7]. The Ornish diet and DASH-type diets (discussed further below) are both consistent with these recommendations and have been shown to improve CVD risk [51,154–160]. However, low-fat diets may be difficult to maintain for many individuals, and other studies have found that reducing fat intake may not translate to a reduction in CVD risk despite modest improvements in intermediate risk factors [14].

CHO restriction has become a popular alternative to low-fat diets, and low-CHO diets have demonstrated some success for weight loss (short term) and other CVD risk factors [161]. For instance, in a recent meta-analysis of 17 RCTs of obese individuals, consuming low-CHO diets for 3–36 months (6–12 month duration of follow-up for majority of studies) significantly increased HDL-C (1.73 mg/dL) and reduced body weight (−7.04 kg), body mass index (BMI) (−2.09 kg/m^2), systolic and diastolic BP (−4.81 and −3.1 mm Hg, respectively), TG (−29.71 mg/dL), fasting glucose (−1.05 mg/dL) and insulin (−2.04 μIU/mL), and CRP (−0.2 mg/L) [161]. Restricting dietary CHO has also been recommended as a primary treatment strategy for type 2 diabetes [162]. However, concerns have been raised that low-CHO diets may result in lower intake of fiber-rich fruits, vegetables, and whole grains (all of which are recommended for CVD risk reduction) and increase the intake of animal protein, SFA, and cholesterol—ultimately increasing CVD risk and mortality [163]. Comparisons of low-CHO diets and low-fat diets [164,165] or isoenergetic balanced diets [166] have also questioned whether low-CHO diets are preferable for CVD risk reduction. For instance, in a meta-analysis of five RCTs of overweight or obese participants, low-CHO diets produced more favorable changes in TG (−22.1 mg/dL) and HDL-C (+ 4.6 mg/dL), while low-fat diets were more effective for TC and LDL-C [164]. The additional weight loss achieved by low-CHO diets in the first 6 months was also no longer present after 1 year [164]. A subsequent 2016 meta-analysis of healthy participants from 11 RCTs found that low-CHO diets produced greater improvements in body weight (−2.17 kg), TG (−23 mg/dL), and HDL-C (+5.4 mg/dL) but also increased LDL-C (+6.2 mg/dL) compared to low-fat diets [165]. However, CHO restriction does not typically occur in isolation and it is likely that the type and/or quality of macronutrient replacement will influence outcomes. Replacing high GI CHO with unrefined plant-based CHO and foods that provide more healthy fats and/or lean protein can be an effective strategy for reducing CVD risk. However, longer term studies with hard endpoints are needed to clarify the effect of consuming a low-CHO diet on CVD risk and mortality.

4 Added Sugars

Added sugars are caloric sweeteners added to foods/beverages during processing or preparation, and do not include naturally occurring sugars such as those in fruit and milk. Added sugars are considered "empty calories" as they provide no essential nutrients and contribute excess calorie intake without improving the overall quality of the diet. Sugar-sweetened beverages (e.g., soft drinks, fruit drinks, sweetened coffee/tea, energy drinks, alcoholic beverages, and flavored waters) are the major source of added sugars in the U.S. diet [7]. Snacks and sweets, including grain-based and dairy-based desserts, are also a primary source. The 2015–2020 DGA recommends that added sugars be limited to less than 10% of daily calories [7]. The 2013 AHA/ACC Guideline also recommends reducing the consumption of sweets and sugar-sweetened beverages, but does not specify added sugars [8].

The recommendation to limit the consumption of added sugars is based largely upon evidence from prospective cohort studies. There is growing evidence that greater intake of added sugars—or sugar-sweetened beverages in particular—increases CVD risk [167], and that individuals who consume higher amounts are more likely to have CVD risk factors, such as obesity, type 2 diabetes, dyslipidemia, and hypertension [168,169]. For instance, numerous prospective studies have demonstrated a significant correlation between the consumption of sugar-sweetened beverages and weight gain [104,170–172]. Conversely, reducing sugar-sweetened beverage intake or replacing sugar-sweetened beverages with water is associated with lower long-term weight gain [104,170]. Fructose in particular—which is a major component of table sugar, high-fructose corn syrup, and other commonly used added sugars—has been shown to dose-dependently increase fasting TGs at intakes >100 g/day [173]. Fructose may also adversely affect visceral adipose deposition and insulin sensitivity [174,175]. Additionally, a recent analysis of National Health and Nutrition

Examination Survey (NHANES) data from 1988 to 2006 found a significant relationship between added sugar consumption and increased risk for CVD mortality [176]. In this study, compared to those who consumed less than 10% of calories from added sugar, individuals who consumed 10−24.9% of calories from added sugar or >25% of calories from added sugar had 30% and 175% greater risk of CVD mortality, respectively [176]. Evidence from RCTs investigating added sugar intake and health outcomes is relatively limited compared to the epidemiological evidence due to cost and feasibility considerations [169]. However, there is evidence from short-term clinical studies to suggest that the mechanistic link between sugar-sweetened beverage consumption and CVD risk may be due to decreased satiety, incomplete compensatory reduction in energy intake at subsequent meals, and/or high GL [168]. Thus, consuming a dietary pattern that includes fewer foods containing added sugars reduces CVD risk and improves multiple CVD risk factors [169].

C Dietary Protein

Increased consumption of dietary protein may benefit cardiovascular health by aiding in weight loss/maintenance, improving the lipid/lipoprotein profile, and reducing blood pressure [177−179]. However, as with other macronutrient substitutions, evidence demonstrating the health benefits of increasing dietary protein must be interpreted with caution. Greater consumption of protein/protein-rich foods typically results in other changes in the diet (i.e., energy, nutrients, and foods) because protein is provided in many different foods. This is likely responsible for the disparate findings regarding the effect of dietary protein intake [180]. For instance, in middle-aged Swedish women [115] and adults in the Greek component of the European Prospective Investigation into Cancer and Nutrition [116] prolonged consumption of a low-CHO high-protein diet has been associated with higher mortality. Conversely, in U.S. women, lower CVD risk was associated with higher protein intake (up to 24% of total calories) [117] or a low-CHO high-protein diet [118]. It is likely that the specific protein food source and the particular macronutrient being replaced in each population influences the effect of increased protein consumption.

With respect to different food sources of protein, there is growing interest in the potential differential effects of plant-based versus animal-based protein on CVD risk [180]. Epidemiological studies have focused primarily on BP. Although numerous epidemiological studies have been conducted, current evidence remains inconsistent. Systematic reviews have found that an inverse association is more commonly found for plant protein but that the current evidence remains limited by inconsistent findings and potential confounders inherent to observational studies [119,181−183]. In studies of industrialized populations, plant protein consumption and blood pressure are more likely to be inversely related, whereas total and animal protein tend to have either no relationship or a direct association with blood pressure [184−186]. Conversely, studies of rural Japanese and Chinese populations have found the opposite relationship, with consumption of animal protein and blood pressure being inversely related [187−189].

The role of plant versus animal protein in cardiovascular outcomes is similarly complex. Analyses of particular food sources of protein (e.g., red meat, poultry, fish, dairy, eggs, legumes, nuts, beans, etc.) have demonstrated that each is associated with a different degree of both CHD risk [190] and CVD mortality [191]. Substituting one standard serving of red meat (~3 oz.) with different plant protein sources was found to reduce CHD risk by 13−30% in the Nurses' Health Study [190] and by 7−19% in combined analyses of the Nurses' Health Study and Health Professionals Follow-up cohorts [191]. It is also necessary to differentiate between various types of red meat to accurately evaluate risk. In a 2010 meta-analysis, Micha et al. concluded that only processed red meat intake, not total red meat intake, was associated with 42% greater risk of CHD [192]. Although dietary guidelines typically recommend reducing high fat red meats due to SFA and cholesterol content, red meat is not among the top contributors to SFA intake in the United States [193] and the primary difference between unprocessed and processed meat is sodium and nonsalt preservatives. However, in other studies, elevated CVD risk has been found with higher intakes of "red meat in addition to processed meat" or total red meat, suggesting that the greater risk associated with red meat is not entirely explained by the salt/preservative content of processed meat [190,194,195]. Therefore, specificity in characterizing each type of plant and/or animal protein is needed to accurately assess associations with CVD risk factors, morbidity, and mortality. The 2015−2020 DGA recommends a variety of nutrient-dense animal and plant sources of protein, with protein providing 10−35% of total calorie intake.

D Other Dietary Components

1 Dietary Cholesterol

Cholesterol is an integral component of cell membranes and is required for steroid hormone and bile acid biosynthesis. However, there is no biological requirement for dietary cholesterol because all tissues synthesize sufficient amounts of cholesterol to meet metabolic and structural needs. Previous dietary guidelines have recommended that cholesterol intake be limited to ≤300 mg/day

because of the dose−response relationship between dietary cholesterol and total and LDL-C [17,196]. However, the epidemiological evidence for a relationship between cholesterol-rich foods such as eggs and CVD risk is inconsistent [197−199]. Furthermore, the hypercholesterolemic effects of dietary cholesterol are relatively small compared to those of saturated fat [17,200], and also extend to HDL-C [201]. Based on this evidence, some have challenged the recommendation to limit dietary cholesterol [201]. However, others continue to assert that dietary cholesterol should be limited, particularly in high-risk individuals, due to harmful effects on postprandial lipemia and LDL-C oxidation [202,203]. The 2015−2020 DGA no longer includes a specific quantitative limit for dietary cholesterol but maintains that dietary cholesterol intake should be kept as low as possible [7]. Because cholesterol and SFA are highly correlated and often found in combination in the same foods [204], following a healthy dietary pattern that is low in SFA is likely to ensure that dietary cholesterol intake is also limited.

2 Plant-Based Bioactives

Although there is substantial epidemiological evidence that fruit and vegetable intake is associated with reduced risk of CVD [159,160,205,206], the mechanisms underlying these health benefits are not fully understood. In addition to fiber and numerous vitamins and minerals, fruits and vegetables also provide phytosterols—the plant analog of cholesterol—and other phytochemicals (i.e., bioactive nonnutritive compounds found in fruits, vegetables, whole grains, and other plant foods). It is likely that each component of this whole food matrix contributes protective effects, and the health benefits of fruits and vegetables cannot be attributed to a single dietary compound. For instance, phytosterols are known for their cholesterol-lowering effects [207−209]. Phytochemicals—which can be classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds [210]—have potent antioxidant activity [211,212]. As oxidative stress is a key driver in the development of many chronic diseases, it is likely that phytochemicals contribute to the health benefits associated with fruit and vegetable intake [213−218]. More than 5000 individual phytochemicals have been identified, but it is thought that a large percentage remain uncharacterized. Supplementation with an isolated micronutrient (i.e., vitamin E, vitamin C, and/or beta-carotene) has been largely unsuccessful in intervention trials [219−225] and it is likely that the beneficial effects of fruits and vegetables do not rely on these well-known antioxidants, but rather on lesser characterized and/or unidentified bioactives, or a concerted action of multiple compounds. The combination of phytochemicals in a particular fruit or vegetable is critical [213], and the consumption of fruits in combination may further enhance antioxidant activity [210]. Outside of the whole food matrix, a purified phytochemical may lose its bioactivity or behave in a different manner. Thus, a whole-food approach that emphasizes the consumption of a variety of fruits and vegetables within a healthy dietary pattern will provide numerous bioactive compounds that may have additive and/or synergistic effects and result in greater CVD risk reduction.

III EVIDENCE-BASED DIETARY PATTERNS FOR REDUCING CVD RISK

The recommendations issued in the 2015−2020 DGA and 2013 AHA/ACC Guideline focus on dietary patterns that are designed to reduce the risk of CVD and other chronic diseases. A whole food, dietary pattern approach guarantees the provision of a wide array of nutrients—many of which have yet to be characterized. Although dietary pattern recommendations typically focus on LDL-C and BP targets, they also address a wide array of other CVD risk factors—ultimately providing greater health benefits than focusing solely on individual nutrients. For example, the recommendation to increase fruit and vegetable intake relates to both hypocholesterolemic and blood pressure-lowering effects of fiber, as well as the potential for phytochemicals to ameliorate oxidative stress and inflammation. Modifications to the type and amount of macronutrients remain a primary goal within this approach to dietary guidelines, but it is important to keep the overall dietary pattern in mind as the effects of any macronutrient change depend in large part on the type of fat, carbohydrate, or protein that is used as a replacement.

There is a strong evidence base for the beneficial effects of the DASH diet, Mediterranean-style diets, and certain vegetarian diets (e.g., Portfolio diet and Ornish diet) [7]. Each of these dietary patterns have unique features, but all emphasize greater intake of nutrient-dense foods (e.g., vegetables, fruits, whole grains, nuts, legumes, unsaturated oils, low-fat dairy, and lean protein) in place of foods high in SFA, TFA, added sugars, and refined CHO. Fig. 27.5 summarizes the key recommendations of the DASH diet, Mediterranean-style diets, and the USDA food patterns recommended by the 2015−2020 DGA.

A DASH Diet

The DASH diet recommends increased consumption of fruits, vegetables, low-fat dairy, whole grains, poultry, fish, and nuts while limiting intake of red meat and added sugars [157]. It is relatively low in total fat (27−28% of total calories), saturated fat (∼6% of total calories), and sodium (2300 mg/day). There is substantial evidence that

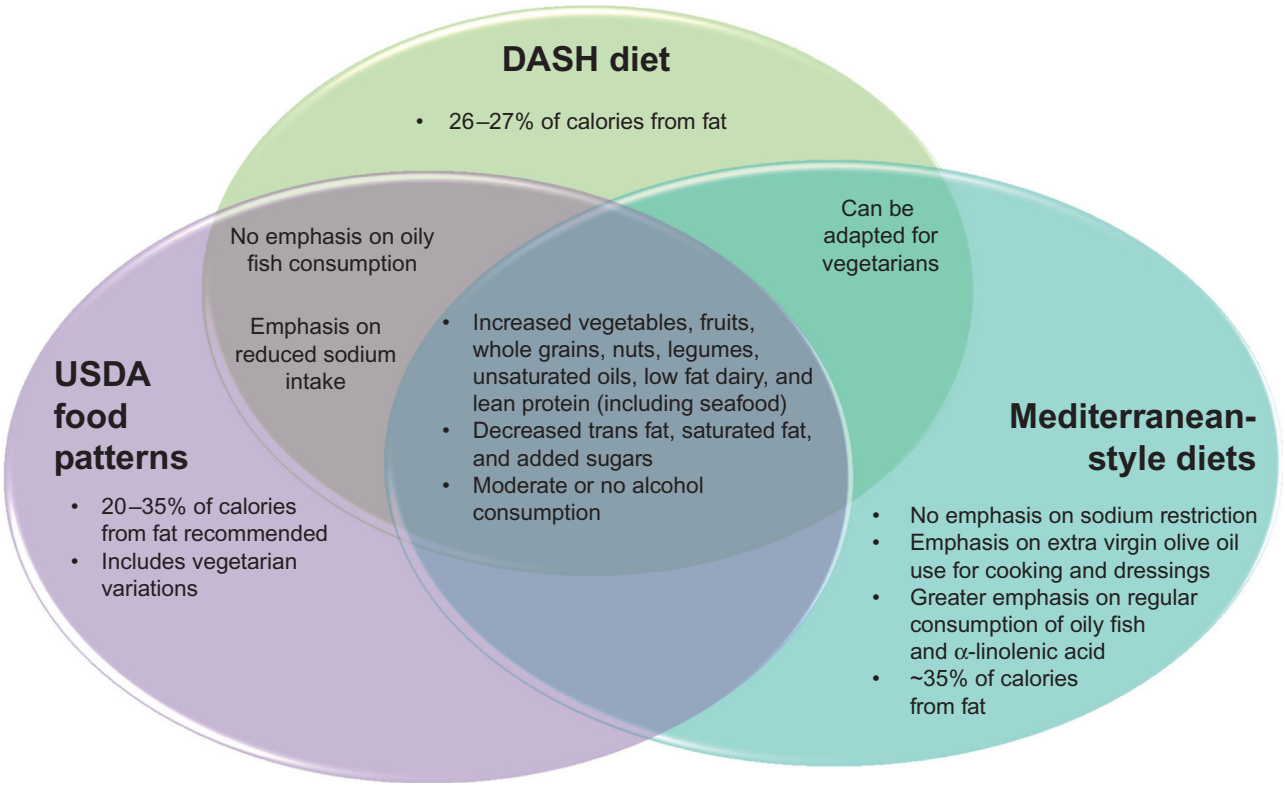

FIGURE 27.5 Commonalities and unique features of the DASH diet, Mediterranean-style diets, and the USDA food pattern recommended by the Dietary Guidelines for Americans 2015. Source: *C.K. Richter, A.C. Skulas-Ray, P.M. Kris-Etherton, Recent findings of studies on the Mediterranean diet: what are the implications for current dietary recommendations? Endocrinol. Metab. Clin. North Am. 43 (4) (2014) 963–980.*

the DASH diet consistently reduces BP, TC, and LDL-C; however, it also reduces HDL-C and does not affect TG [8]. In the original DASH trial [157, 158], participants who consumed the DASH diet had significantly greater reductions in TC (−13.7 mg/dL), LDL-C (−10.7 mg/dL), HDL-C (−3.7 mg/dL), and BP (−5.5/−3.0 mm Hg) compared to individuals consuming the typical American diet control. It was estimated that this translated to an 18% reduction in 10-year CHD risk according to the Framingham risk equations [227].

Additional sodium restriction in the context of a DASH diet can also provide further cardiovascular benefit. In the DASH-Sodium Trial, adults with elevated BP ($n = 412$) were randomized to either a typical American diet or the standard DASH diet. Within their assigned diet group, participants consumed foods to achieve a high, moderate, or low sodium intake for 30-day periods in a randomized crossover design (approximately 3450, 2300, and 1150 mg/day per 2100 kcal, respectively) [228]. The high and intermediate sodium levels were designed to reflect the typical intake in the United States and the upper limit of national recommendations, respectively. Sodium reduction dose-dependently lowered systolic and diastolic BP across all subgroups (i.e., sex, ethnicity, and hypertensive status) with both the control diet and the DASH diet; however, effects were greater in participants who were older [229] and who had hypertension [228]. Compared to the control diet, the DASH diet produced significantly lower systolic BP at each sodium level [228], and the combination of the two interventions—sodium restriction and the DASH diet—provided the greatest benefit. Sodium intake did not affect blood lipid concentrations [230]. Thus, reducing sodium intake—either to current recommendations or to a still lower level of intake—lowers BP and is an important dietary intervention for CVD risk reduction, even in the context of a typical American diet.

Improvements in CVD risk factors can also be achieved using a DASH diet with a modified macronutrient profile. The Optimal Macronutrient Intake Trial to Prevent Heart Disease (OmniHeart) was designed to test the effect of replacing 10% of the CHO in a higher CHO DASH diet (58% CHO, 15% protein, and 27% fat) with either protein or unsaturated fat. All three dietary interventions had favorable effects on BP, lipids, and 10-year CHD risk [231]. However, compared to the high-CHO DASH diet, both the high-protein and unsaturated fat DASH diet variations resulted in significantly greater reductions in systolic and diastolic BP, and lowered TG

[231]. Furthermore, the high-protein DASH diet also reduced LDL-C significantly more than the high-CHO DASH and the unsaturated fat DASH diet increased HDL-C [231]. Therefore, modifying the macronutrient profile of the standard DASH diet can result in greater CVD risk reduction, and these DASH diet variations provide additional options for implementing a healthy dietary pattern.

Systematic reviews of subsequent RCTs using a DASH-style dietary intervention have found similarly beneficial effects on BP, lipids/lipoproteins, and other CVD risk factors. For instance, in a 2015 meta-analysis of 20 RCTs comprising 1917 participants, a DASH diet produced significant reductions in systolic BP (−5.2 mm Hg), diastolic BP (−2.6 mm Hg), and LDL (−3.9 mg/dL) [232]. However, BP reductions were greater in participants with elevated baseline BP or BMI. Together, these changes in BP and cholesterol translated to a 13% reduction in the estimated 10-year Framingham risk score for CVD. Systematic reviews focused specifically on the BP effects of DASH diet interventions have reported similar findings [233,234]. A DASH-style diet may also be beneficial for body weight. Among 10 studies assessing the effect of a DASH diet on body weight, the average weight reduction was 1.42 kg for interventions lasting 8−24 weeks [235]. The reduction in body weight was greater when the DASH diet was implemented as part of an energy-restricted diet (−2.27 kg), for less than 12 weeks (−1.64 kg), and in overweight/obese participants (−1.63 kg) [235]. Therefore, there is strong evidence for the cardiovascular benefits of DASH-diet interventions.

The long-term effectiveness of adherence to a DASH diet is less well defined and relatively few studies have investigated effects on disease incidence. However, a systematic review of six studies concluded that a DASH-style diet significantly reduced the risk of CVD, CHD, stroke, and heart failure incidence by 20%, 21%, 19%, and 29%, respectively [236]. Many studies have reported an inverse relationship between DASH-diet compliance and blood pressure or incident hypertension [237−240], but not all results have been consistent [241,242]. For instance, in a cross-sectional analysis of 2047 adults, self-reported adherence was inversely related to systolic BP, with a difference of 4.4 mm Hg for participants with the highest DASH diet compliance score compared to participants with the lowest [237]. Conversely, in the Framingham Offspring cohort and the Iowa Women's Health Study, DASH diet adherence was not inversely associated with BP or hypertension risk [241,242]. However, it should be noted that participants' adherence to the DASH diet was relatively low in both of these studies, and few participants met the recommendations for specific food groups. The long-term benefits of the DASH diet for CVD risk reduction likely depend on the level of adherence to dietary targets.

There is a large body of evidence demonstrating that a DASH-style diet improves multiple CVD risk factors and reduces the risk of developing CVD. However, compliance to DASH-diet recommendations may be harder to achieve in "real world" settings compared to trials in which foods are provided to participants, and additional approaches may be needed to help individuals implement and sustain a DASH-style dietary pattern. For instance, the ability to modify the standard DASH diet and maintain or enhance its beneficial effects can provide greater flexibility in accommodating personal preferences. The DASH diet is the primary dietary pattern recommended by the 2013 AHA/ACC Guideline for the reduction of LDL-C and BP. Table 27.5 provides a summary of the BP and lipid effects of the diet recommendations in the 2013 AHA/ACC Guideline (i.e., standard DASH diet, DASH-diet variations, and sodium restriction).

B Mediterranean-Style Diets

A Mediterranean-style dietary pattern represents the food habits of the countries in the broad geographical area surrounding the Mediterranean Sea. Although there are country-specific variations in the foods consumed, a Mediterranean-style diet generally emphasizes fresh fruits, root and green vegetables, grains (mostly whole), legumes, oily fish rich in omega-3 FAs, nuts, seeds, and olive oil in place of butter or other animal-based fats. Low-fat or fat-free dairy is consumed daily; fish, poultry, and eggs are consumed in low to moderate amounts; and red meat and sweets are limited. Wine is also consumed in low to moderate amounts in non-Islamic countries. Overall, a Mediterranean-style diet tends to be moderate in total fat (32−35% of total calories), relatively low in saturated fat (9−10% of total calories), high in fiber (27−37 g/day), and high in MUFAs and PUFAs.

The benefits of a Mediterranean-style dietary pattern for CVD risk are based on both epidemiologic evidence and clinical intervention studies. Interest in the Mediterranean-style diet was first generated by reports from the Seven Countries Study in the 1980s, which found that the 15-year mortality rate from CVD in the Mediterranean region of southern Europe was 2−3 times lower than in northern Europe and the United States [243]. It was hypothesized that this was due to the dietary habits of these populations as regions with the highest CVD mortality also had greater SFA intake and higher serum cholesterol. Subsequently, the Lyon Diet Heart Study [111,112] was designed to test the effects of a Mediterranean-style diet on secondary prevention of MI. Participants were randomized to consume either the prudent Western-style control diet or a Mediterranean-style diet lower in SFA and higher in ALA for 104 weeks. Participants who consumed the Mediterranean-style diet

TABLE 27.5 Summary of Diet Recommendations Issued in the 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk and Effects on Blood Pressure and Lipids

Modification	Recommendation	Systolic and Diastolic Blood Pressure Reduction[a] (*Strength of Evidence*)	Lipid Effects[a] (*Strength of Evidence*)
DASH diet[b]	Consume a diet high in vegetables, fruits, low-fat dairy products, whole grains, poultry, fish, and nuts; low in sweets, sugar-sweetened beverages, and red meats	5−6/3 mm Hg (*High*)	↓ LDL-C by 11 mg/dL ↓ HDL-C by 4 mg/dL ↔ TG (*High*)
DASH variations[c]	DASH dietary pattern with 10% of total daily energy from CHO replaced with protein	1 mm Hg (systolic) (*Moderate*)	↓ LDL-C by 3 mg/dL ↓ HDL-C by 1 mg/dL ↓ TG by 16 mg/dL (*Moderate*)
	DASH dietary pattern with 10% of total daily energy from CHO replaced with unsaturated fat (8% MUFA, 2% PUFA)		Similar LDL-C reduction as DASH ↑ HDL-C by 1 mg/dL ↓ TG by 10 mg/dL (*Moderate*)
Dietary sodium restriction	Reduce sodium intake by at least 1000 mg/day; consume no more than 2400 mg/day; further reduction to 1500 mg/day is desirable	2/1 mm Hg with 2400 mg sodium 7/3 mm Hg with 1500 mg sodium (*Moderate*)	–

[a]*Strength of the evidence-base for each recommendation as graded by AHA/ACC Work Group given in parentheses.*
[b]*Compared to typical 1990s American diet.*
[c]*Compared to DASH dietary pattern (i.e., additional reductions in BP and lipids, relative to those achieved by standard DASH dietary pattern).*
BP, blood pressure; DASH, Dietary Approaches to Stop Hypertension; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides.

had similar BMI and BP compared to the control group and experienced no change in blood lipids, but had markedly lower prevalence of cardiac death and nonfatal MI, fewer major secondary events, and decreased hospitalizations [112]. Overall, subjects consuming the Mediterranean-style diet had a 50−70% lower risk of recurrent cardiac events, and the study was stopped early because of the evidence of significant CVD benefit [112]. It has been suggested that the abundance of bioactive compounds in the Mediterranean-style diet may in part account for these CVD outcomes, potentially via favorable effects on vascular function, arrhythmia, and oxidative stress.

Observational studies have also demonstrated the beneficial effects of a Mediterranean-style diet on CVD. Meta-analyses have consistently reported that greater adherence to a Mediterranean-style diet is associated with significant reduction in CVD risk, morbidity, and/or mortality [56,244−249]. For instance, in an analysis of 81,722 women in the Nurses' Health Study, women in the highest quintile for the Mediterranean diet score (high intake of vegetables, fruits, nuts, whole grains, legumes, and fish; high ratio of MUFAs to SFAs; moderate intake of alcohol; and low intake of red and processed meat) experienced a 40% reduction in sudden cardiac death relative to women consuming a diet that was least reflective of this pattern [247]. A 2015 systematic review of 24 observational studies reported that individuals in the highest quintile of adherence had lower CVD incidence (RR: 0.73; 95% CI: 0.66−0.80) and mortality (RR: 0.75; 95% CI: 0.68−0.83) compared to those who were least adherent [249]. Significant risk reduction was also found for the incidence of CHD (RR: 0.72; 95% CI: 0.60−0.86), MI (RR: 0.67; 95% CI: 0.54−0.83), and stroke (RR: 0.76; 95% CI: 0.60−0.96) [249]. A Mediterranean-style diet may also reduce the risk of type 2 diabetes and metabolic syndrome [250,251]—both of which increase CVD risk. With regard to diabetes, a meta-analysis of seven cohort studies found a 27% reduction in relative risk for highest versus lowest adherence to the Mediterranean diet score [251]. Thus, there is strong epidemiological evidence for the cardiovascular benefits of consuming a Mediterranean-style dietary pattern.

Multiple short-term intervention trials have also evaluated the effects of a Mediterranean-style diet on CVD risk factors [226]. Overall, meta-analyses of these

RCTs have found significant improvements in lipids/lipoproteins, BP, endothelial function, insulin resistance, and inflammation with Mediterranean-style dietary interventions [252−255]. The lack of significant CVD benefit found in some studies [112,256−259] may reflect differences in study design, duration, and the type of control/comparator diet. However, it should be noted that many of these studies used a parallel arm design and the type of Mediterranean-style diet intervention (and the control or comparator diet) varied greatly among many of these studies.

There are relatively limited data regarding the effect of a Mediterranean-style dietary pattern on CVD morbidity and mortality. Prior to the mid-2000s, the Lyon Diet Heart Study was the only clinical trial that evaluated the effect of a Mediterranean-style diet on cardiovascular morbidity and mortality. In 2003, the Prevención con Dieta Mediterránea (PREDIMED) study—a multicenter clinical trial evaluating the efficacy of a Mediterranean-style diet for primary prevention of CVD—was started. Participants were 55−80 years of age with type 2 diabetes or ≥3 major CVD risk factors (hypertension, hypercholesterolemia, family history of heart disease, tobacco use, or overweight/obesity) and were randomized to: (1) a low (er)-fat diet, (2) a Mediterranean diet with extra-virgin olive oil (1 L/week/family; 50 g/day per participant), or (3) a Mediterranean diet with tree nuts (30 g/day: 15 g walnuts, 7.5 g hazelnuts, 7.5 g almonds) [260,261]. After 3 months, subjects in both Mediterranean diet groups had lower TC/HDL-C ratios compared to the low(er)-fat group (−0.38 and −0.26, respectively) [260]. Additionally, the Mediterranean diet with nuts reduced fasting glucose (−5.4 mg/dL), systolic BP (−7.1 mm Hg), diastolic BP (−2.6 mm Hg), and TG concentrations (−13 mg/dL) relative to the low(er)-fat diet [260]. The Mediterranean diet with olive oil also reduced fasting glucose (−7.0 mg/dL), SBP (−5.9 mmHg), and DBP (−1.6 mmHg), but did not reduce TG concentrations compared to the low(er)-fat diet [260]. The trial was stopped early because of the significant clinical benefits observed for the combined endpoint of stroke, MI, or death from cardiovascular outcomes [261]. Participants in both the higher nut and higher extra-virgin olive oil diets experienced an approximate 30% reduction in the primary endpoint relative to the low(er) fat diet group [261]. When the individual components of the combined CVD outcome were analyzed separately, only the reduction in stroke risk reached significance (HR for Mediterranean diet plus olive oil: 0.67 [95% CI: 0.46−0.98], and 0.54 [95% CI: 0.35−0.84] for Mediterranean diet plus nuts). The Mediterranean diet with olive oil also reduced the incidence of new-onset type 2 diabetes compared to the control diet [262]. It is important to note that the macronutrient composition of the three diet treatments was quite similar, with only a 4 percentage-point difference in total fat intake between the low(er) fat and Mediterranean diet groups. The Lyon Diet Heart Study and PREDIMED remain the only long-term intervention studies of a Mediterranean-style dietary pattern, and demonstrate that this dietary pattern can significantly improve multiple CVD risk factors and reduce cardiovascular mortality.

A Mediterranean-style dietary pattern is recommended by both the 2015−2020 DGA and the AHA/ACC Guideline for lowering CVD risk. According to the AHA/ACC grading methodology, the strength of evidence for the LDL-C and BP-lowering effects of a Mediterranean dietary pattern is "low." However, this is due to the fact that the scope of the AHA/ACC Guideline was limited to modifiable CVD risk factors (e.g., lipids/lipoproteins and blood pressure) rather than cardiovascular events [8]. As such, the 2013 AHA/ACC Guideline did not review the evidence for morbidity and mortality in regards to dietary patterns and does not include the most recent results from PREDIMED [263].

C Vegetarian Diets

Vegetarian diets emphasize fruits, vegetables, whole grains, legumes, nuts, seeds, and soy foods and include little to no animal products. Compared to nonvegetarians, vegetarians typically consume fewer calories, SFA, cholesterol, and sodium but more fiber and total CHO [264,265]. As a whole, these factors can impart favorable effects on CVD risk factors. However, individuals choosing to omit all animal products from their diets must be well informed to ensure adequate intake of some nutrients. A vegetarian diet that is poorly planned with respect to nutrient recommendations will not decrease CVD risk and may increase the potential for nutrient deficiencies. The 2015−2020 DGA includes a healthy vegetarian-style eating pattern but different types of vegetarian diets may vary in the specific food groups that are restricted (e.g., eggs and dairy). The two vegetarian diets with the largest evidence base are described in detail below.

The Portfolio Diet is a vegetarian diet designed to achieve maximal LDL-C reduction. The diet is very low in SFA and cholesterol, and is characterized by its combination of plant sterols, viscous fibers (primarily from oat, barley, and psyllium), soy protein (21 g/1000 kcal), and almonds (14 g/1000 kcal)—the four functional foods recommended by the AHA and National Cholesterol Education Panel (NCEP) Adult Treatment Panel (ATP) III for their cholesterol-lowering capacity [209,266]. In short-term intervention studies, LDL-C reductions of 28−35% have been achieved [209,266−268]. For instance, under controlled feeding conditions, following a Portfolio diet (20% of calories as vegetable protein,

50.6% CHO, 23.2% fat [4.9% SFA, 9.5% MUFA, and 7.9% PUFA], 48 mg cholesterol per 1000 kcal, and 37.2 g fiber per 1000 kcal) for 4 weeks reduced TC and LDL-C (26.6% and 35.0%, respectively) in hyperlipidemic subjects, with no significant effect on HDL-C or TG [266]. These reductions were significantly greater than those produced by the low-SFA diet control. In a crossover study comparing the effects of the Portfolio Diet to a low-SFA control and a low-SFA diet plus statin treatment, the Portfolio Diet reduced LDL-C by 20.6% after 1 month [268]. Although this was significantly different than the effect of the statin treatment (−33.3% LDL-C), the Portfolio Diet and statin treatment were similarly effective in reducing LDL-C to below the 130 mg/dL target [268].

Under free-living conditions, the Portfolio Diet has also been shown to improve LDL-C and other CVD risk factors, although these changes tend to be more modest. In a 1 year free-living study of hypercholesterolemic adults ($n = 66$), maximum LDL-C reduction occurred at 12 weeks (−14%), and these benefits were sustained to the 1 year follow-up (−12.8%) [269]. Approximately one-third of these participants experienced LDL-C reductions of ≥20%, which is similar to the effect of first generation statin treatment. Self-reported dietary compliance—in terms of plant sterol, viscous fiber, soy protein, and nut intake—over the 1 year study period was significantly correlated with the change in LDL-C ($r = -0.42$; $p < 0.001$). However, improvements in LDL-C were also achieved without complete adherence to other portions of the dietary recommendations. For instance, after 1 year, only 2 of 55 participants were following a vegan diet, and five were following a lacto-ovo-vegetarian diet. The remaining participants returned to an omnivorous diet [269]. Similar LDL-C reductions (−13%) were achieved in a subsequent free-living study in which participants received counseling to follow the Portfolio Diet for 6 months [270]. This was significantly more than the reduction achieved by low-SFA dietary advice control (−3%) [270]. Effects of the Portfolio Diet on other CVD risk factors are less well established, but there is some evidence for BP reduction [271,272]. The Portfolio Diet has also been shown to lower the inflammatory marker CRP to a similar extent as statin therapy—an effect that has not been achieved by conventional cholesterol-lowering diets [267,273].

Modified Portfolio Diets have also been developed to target additional CVD risk factors. For instance, substituting 13% of the total calories from CHO with MUFA has been tested under controlled feeding conditions. After 1 month, hyperlipidemic patients randomized to either a low-MUFA (13% of total kcal) or high-MUFA (26% of total kcal) Portfolio Diet experienced similar reductions in LDL-C (−35%), but the high-MUFA Portfolio Diet also increased HDL-C and lowered CRP compared to the low-MUFA control [274]. Increasing the MUFA content of the Portfolio Diet also increased the apoA1 pool size and tended to increase LDL clearance rate ($p = 0.09$) relative to the low-MUFA comparator [275]. Adding strawberries to the standard Portfolio Diet has also been shown to provide greater protection against LDL oxidation while maintaining reductions in LDL-C (−13.4%) and the TC:HDL-C ratio (−15.2%) [276].

The Ornish diet is another plant-based diet that has been shown to produce striking reductions in CVD risk factors and events. The Ornish diet advocates "intensive lifestyle changes" and incorporates aerobic exercise, meditation, and smoking cessation in addition to dietary modifications. The diet is characterized by the exclusion of animal products (except for minimal amounts of nonfat yogurt), salt, alcohol, sugar, and caffeine; being very low fat (<10% of total calories); and emphasizing fresh fruits and vegetables, whole grains, legumes, tubers, and soybean products. All sources of fat are restricted, including vegetarian sources such as avocados, seeds, and oils, and questions have been raised about the lack of distinction between different types of fats. An unfavorable FA profile is unlikely when following a low-fat, whole-food vegetarian diet but omega-3 FAs may need to be obtained from supplements and/or enriched food products. In the Lifestyle Heart Trial, 48 patients with moderate to severe CVD were randomized to the Ornish diet or a usual care control group. Under short-term inpatient conditions, the Ornish diet improved left ventricular response to exercise and reduced TC (−20.5%) and anginal episodes (−91%) [156]. The reductions in LDL-C and angina episodes were maintained by patients in the Ornish group after 1 year of free-living conditions [154]. After 5 years, 35 participants remained in the study with similar compliance between groups (71% of intervention patients and 75% of control patients). Patients following the Ornish diet experienced more regression in coronary artery stenosis and fewer cardiac events compared to the standard-care control group [155]. The LDL-C reductions of 37% at 1 year [154] and 20% after 5 years [155] are comparable to the effects of statin therapy.

Since the Lifestyle Heart Trial, subsequent studies have provided further support for Ornish lifestyle interventions. For instance, in CVD patients, the Ornish program significantly reduced anginal frequency, body weight, BMI, systolic BP, TC, LDL-C, and glucose compared to traditional cardiac rehabilitation and a control group that received no formal cardiac risk reduction [277]. Similar benefits for CVD risk factors were reported for patients with coronary artery disease [278−280], although the Ornish program had no significant effect on carotid intima-media thickness compared to traditional cardiac rehabilitation [280]. An ongoing Multisite Cardiac Lifestyle Intervention Program for primary and secondary

prevention of CHD has reported significant reduction in overall coronary risk after 3 months [281] by addressing multiple CVD risk factors, including endothelial function and inflammatory markers [282], as well as BMI, systolic and diastolic BP, and LDL-C [283]. However, it should be noted that most of these changes are relative to baseline values rather than a control group [278,279,282,283]. In other studies, the Ornish diet has also demonstrated significant reductions in LDL-C during both weight loss [284] and weight maintenance [285]. However, the Ornish program also typically reduces HDL-C [284,285].

Despite these promising findings for the beneficial cardiovascular effects of the Portfolio and Ornish diets, additional strategies may be needed to reinforce adherence and achieve maximal CVD risk reduction. For instance, under free-living conditions, overall adherence to the Portfolio Diet was only 46.4% after 6 months despite multiple dietary counseling sessions [270]. Discontinuation rates after 1 year were similarly high (50%) for the Ornish diet in a free-living study [284]. Nonetheless, significant improvements in multiple CVD risk factors were achieved in both cases [270, 284]. The Ornish plan is also covered by Medicare as an alternative intensive cardiac rehabilitation program, and other studies have reported higher retention rates [286]. Adding strawberries to the Portfolio Diet has previously been shown to increase palatability, and the addition of strawberries or other fruits may improve the overall utility of the Portfolio Diet for CVD risk reduction [276]. Additional research is needed to identify practical techniques to increase dietary adherence. For instance, individuals can be matched to a diet that is well-suited to their personal food preferences and lifestyle. Cost and time considerations should also be taken into account to ensure that healthy dietary patterns are achievable for all individuals regardless of financial limitations.

IV CONCLUSIONS

A health-promoting dietary pattern can be achieved in a variety of ways, but consistently emphasizes the consumption of nutrient-dense foods (e.g., fruits, vegetables, whole grains, legumes, oily fish, nuts and seeds, lean meat, low-fat/skim dairy products, and liquid vegetable oils) in place of foods that are high in SFA, TFA, refined CHO, and added sugars. The specific evidence-based dietary patterns discussed in this chapter (i.e., the DASH Diet, Mediterranean-style diet, and vegetarian diets) each have characteristic features, but can be modified with respect to macronutrient distributions to suit an individual's specific needs and preferences. It is likely that the ability to personalize healthy dietary patterns will improve long-term adherence and maximize CVD risk reduction. These dietary patterns are recommended in the 2015–2020 DGA and 2013 AHA/ACC Guideline on Lifestyle Management, and can modify both traditional and emerging CVD risk factors. A healthy diet is essential for the prevention and treatment of CVD, and implementation of these evidence-based dietary patterns can have a substantial impact on public health.

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Chapter 28

Nutrition, Lifestyle, and Hypertension

Pao-Hwa Lin, Crystal C. Tyson, Bryan C. Batch and Laura P. Svetkey
Duke University Medical Center, Durham, NC, United States

I INTRODUCTION

Hypertension is a major risk factor for coronary heart disease (CHD), stroke, and premature death and a leading risk factor for global disease burden [1−5]. Prevalence for those 18 years and older in the United States has remained around 29% since 1999 with little improvement [6]. Further approximately one-third of American adults have prehypertension [7], which is also associated with a graded, increased risk of cardiovascular disease (CVD) and progression to hypertension [4,5,8]. Studies have shown that for every 20-mmHg increase in systolic blood pressure (SBP) or 10-mmHg increase in diastolic blood pressure (DBP) there is a doubling of mortality from ischemic heart disease (IHD) and stroke (Table 28.1) [4]. In industrialized societies such as the United States, blood pressure (BP) increases with age: the prevalence of hypertension is 7.3% among adults aged 18−39 years, and the figure increases to 32.3% among those aged 40−59 years and to 64.9% among those 60 years and above [6]. Hypertension is more common in non-Hispanic black (41.2%) than non-Hispanic white (28.0%), non-Hispanic Asian (24.9%), or Hispanic (25.9%) adults. Although the cause of hypertension is largely unknown, 1−5% of hypertension cases are due to a secondary underlying correctable condition.

In contrast to the prevalence in the United States and many developed or developing countries, many non-Westernized, remote populations have a low prevalence of hypertension and do not experience an increase in BP with age [9,10]. Their protection from hypertension is often attributed to very low salt intake [11−13], high potassium intake [12,14], being physically active [9], low alcohol consumption [12,15], and generally high plant food and fish consumption. Migration studies of indigenous populations also report increasing prevalence of hypertension with urbanization, providing additional evidence for a role of environmental (including nutritional) factors [16,17]. With urbanization, access to processed food increases which in turn reduces diet quality in general, increases intakes of the BP raising nutrients like sodium and saturated fat and decreases intakes of BP-lowering nutrients like potassium, magnesium, and fiber. Further fresh foods that were previously readily available become less affordable. These changes potentially contribute to the prevalence of hypertension. In addition to other lifestyle changes, increases in body weight, sodium intake, dietary fat, and the ratio of urinary sodium-to-potassium have been observed during the process of acculturation [15−21]. Taken all together these observations support an important role of diet and lifestyle in BP regulation and inspired much of the later research in this area.

The relationship between dietary intake and BP suggests the possibility of nutritional intervention to prevent the development of hypertension, an urgent need given its high prevalence and associated risk. In addition, effective strategies are urgently needed to improve the poor rates of hypertension control. BP control among adults with hypertension has increased from 31.5% in 1999−2000 to 53.3% in 2013−14, however, there remain nearly half of adults with hypertension with uncontrolled hypertension [6].

In this chapter we provide an overview of epidemiologic and clinical evidence for established and potential dietary factors for hypertension prevention and control. Because a comprehensive review of all individual trials in this area is beyond the scope of this chapter, when applicable, we review meta-analyses. Although meta-analyses are useful for evaluating consistency in the literature, they tend to weigh large studies more heavily [22,23], may be limited by the predetermined inclusion criteria, and it is

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TABLE 28.1 Baseline Systolic blood pressure (SBP) and Age-Adjusted 10-Year Mortality of Cardiovascular Disease (CVD) From the Multiple Risk Factor Intervention Trial

SBP (mmHg)	*n*	Deaths	Rate per 1000	Relative Risk	Excess Deaths	% of All Excess Deaths
<110	21,379	202	10.5	1.0	0.0	0.0
110–119	66,080	658	11.0	1.0	33.0	1.0
120–129	98,834	1324	14.3	1.4	375.6	11.5
130–139	79,308	1576	19.8	1.9	737.6	22.6
140–149	44,388	1310	27.3	2.6	745.7	22.8
150–159	21,477	946	38.1	3.6	592.8	18.2
160–169	9308	488	44.8	4.3	319.3	9.8
170–179	4013	302	65.5	6.2	220.7	6.8
≥180	3191	335	85.5	8.1	239.3	7.3

Note: Men free of history of myocardial infarction at baseline (*N* = 3 47,978); Multiple Risk Factor Intervention Trial primary screens. [8]
Source: Reprinted with permission from J. Stamler, BP and high BP: aspects of risk. Hypertension 18 (Suppl 1) (1991) I95–I107.

rarely feasible to conduct subanalyses on potentially important modifying factors or to account for variable dietary adherence among studies. Nonetheless, whenever available, multiple meta-analyses combined provide comprehensive overview of the existing evidence.

In addition to the scientific foundation found in epidemiologic and clinical data, it is important to consider application of these findings. Accordingly, the last two sections of this chapter are devoted to the review of several large-scale intervention trials. These trials include the whole diet-based controlled feeding trials such as the Dietary Approaches to Stop Hypertension (DASH) trial [24], DASH-Sodium [25], and OmniHeart [26] trials. Additionally large-scale multilifestyle intervention trials including Trials of Hypertension Prevention (TOHP) I [27], TOHPII [28], and PREMIER [29] are also reviewed. Issues related to implementation of the current national guidelines are discussed. We conclude with a summary of qualitative and quantitative dietary and lifestyle recommendations for the prevention and treatment of hypertension.

II MICRONUTRIENTS

In observational studies, micronutrients directly associated with lower BP include potassium, magnesium, calcium, and fiber; an inverse association is seen with dietary sodium. Epidemiologic and clinical data that demonstrate these relationships are reviewed further. However, it is important to recognize that determining the independent effect of each micronutrient on BP is often limited because several of these micronutrients derive from similar food sources [30] and are highly correlated with each other. For example, foods high in magnesium (e.g., nuts) are also high in fiber and potassium, making it virtually impossible to attribute associations with BP to the effects of a single micronutrient. To isolate and test the effect of individual nutrients, intervention studies typically use dietary supplements. The use of supplements allows for any changes in BP seen as a result of the intervention to be attributed solely to the nutrient being examined. Although using supplements provides an opportunity for the relationship between an individual micronutrient and BP to be precisely examined, it also has its limitations. Micronutrient supplements may not be absorbed as well or have the same physiologic effects when they are consumed in food. Varying levels of other dietary components may also modify the effectiveness of the supplemented micronutrient. These challenges have led to less focus on individual micronutrients and increased focus on dietary patterns, as discussed later in this chapter.

A Potassium

The evidence for a role of potassium in lowering BP is relatively consistent across study types and is biologically plausible [31].

1 Observational Studies

An extensive body of epidemiologic data demonstrates an association between dietary potassium and BP [32]. Both potassium alone (inversely) and the sodium-to-potassium ratio (directly) have been associated with BP [33,34]. A lower urinary excretion of potassium (a good marker of dietary intake) is associated with higher BP [32] and risk for hypertension [35]. The linear association of BP with

potassium intake is actually tighter than with sodium intake, with the mean potassium intake in hypertensives about 15% lower than in nonhypertensives [36].

2 Interventional Studies

Several meta-analyses have examined the effect of potassium supplementation on BP. In 1997 Whelton et al. [37] reviewed 33 randomized controlled trials (RCT) of potassium and BP. These studies included mostly individuals with hypertension, some of whom were receiving antihypertensive medication. In all studies potassium was provided as a supplement (median 75 mmol (2925 mg)/day), either superimposed on a controlled research diet or added to participants' usual diets. After excluding one outlier study, a high potassium intake was associated with net 3.11/1.97-mmHg reduction in SBP/DBP. Interestingly, greater BP reductions occurred in those with progressively higher urinary sodium excretion during follow-up (measured at the end of the study). This suggests that potassium is more effective at higher levels of sodium intake. Other research evidence also points to the interaction of potassium and sodium in BP [31]. In addition, results were significantly stronger in studies that included a high proportion of African Americans. This meta-analysis reached qualitatively similar conclusions to those of a 1995 analysis by Cappuccio et al. [38] with roughly a 50% overlap in studies included.

In a large intervention trial of US female nurses (Nurses Health Study II), Sacks and colleagues [39] administered either supplemental potassium (40 mmol, 1560 mg), calcium (30 mmol, 1200 mg), magnesium (14 mmol, 336 mg), or all three minerals combined, or placebo to women who reported habitually low intakes of these nutrients, for a 6-month period. Potassium, administered alone, was the only intervention that reduced BP. The mild yet significant reduction occurred even though the women were nonhypertensive (mean BP: 116/73 mmHg).

Furthermore two meta-analyses provide supporting evidence for an effect of a potassium supplement on BP. Geleijnse et al. [40] examined 27 randomized trials with at least 2 weeks of follow-up among both hypertensives and nonhypertensives. They found that a median potassium supplementation of 44 mmol/day (1716 mg/day) was associated with a reduction of SBP/DBP by 2.42/1.57 mmHg. This reduction was even greater among those who were hypertensive (3.51/2.51 mmHg), but it was only borderline significant ($p=0.08$). Dickinson et al. [41] reviewed 4 of the 27 trials in the Geleijnse study plus 2 other trials, all of which included at least 8 weeks follow-up and only hypertensives. They found a large reduction in BP by potassium supplementation (11.2/5.0 mmHg), but the reduction was not statistically significant, perhaps partially because of the heterogeneity of the trials. The authors stated that the heterogeneity of the trials was not explained by the varying dosage of potassium supplementation, study quality, or the baseline BP.

Perhaps the strongest support for the effect of dietary potassium on BP comes from the DASH trial. DASH was a controlled feeding study in which adults with prehypertension or untreated stage I hypertension were randomly assigned to one of three diets: a typical American diet, a diet that eventually became known as "the DASH dietary pattern" (see below), and a fruits and vegetables diet that was identical to the typical American diet except for the addition of more fruits and vegetables. All three treatment groups were fed isocaloric diets without sodium restriction. The nutrient content of the fruits and vegetables diet, relative to the control diet, was increased in potassium, magnesium, and fiber. Participants eating 2000 kcal/day in this study consumed 4700 mg/day of potassium; potassium intake was increased or decreased proportionally for higher or lower energy intakes. The fruits and vegetables diet significantly reduced SBP/DBP, relative to the control diet, by 2.8/1.1 mmHg [24]. Although the increase in potassium was not the only difference from the control diet, the strength of epidemiologic, metaanalytic and biologic evidence suggests that potassium was the BP-active nutrient.

Further, the BP reduction was greater in those with hypertension (11.6/5.3 mmHg) compared to prehypertension (3.5/2.2 mmHg) and among African Americans (6.9/3.7 mmHg) compared to non-African Americans (3.3/2.4 mmHg) [42]. The greater effect in African Americans is particularly intriguing since this population has both higher rates and severity of hypertension [43] and lower dietary intake of potassium [44], suggesting both a mechanism and an intervention for racial disparities in hypertension.

3 Mechanism

Potassium may lower BP through a direct vasodilatory role, alterations in the renin–angiotensin system [45], nitric oxide production [46], renal sodium handling, and/or natriuretic effects [47,48] (Fig. 28.1).

4 Summary

There is compelling evidence that increasing potassium intake lowers BP. Thus research strongly supports the recommendation of increasing potassium intake to 4700 mg/day for the prevention and control of high BP. This recommendation has been endorsed by the American Heart Association (AHA) [49] and Dietary Guidelines for Americans [50]. However, average intake in the United

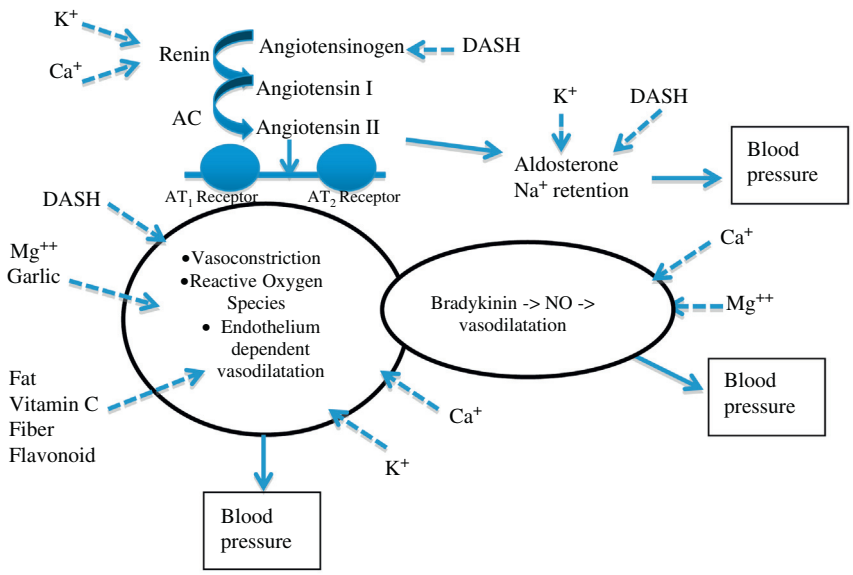

FIGURE 28.1 The potential effect of various dietary factors and DASH dietary pattern on BP regulation.

States continues to fall below this recommendation, with less than 3% of adults consuming 4700 mg/day [51]. Even though potassium is rich in foods of various food groups, choices may need to be intentionally planned in order to reach this recommendation.

B Sodium

Many studies have supported the relationship between sodium intake with BP, CVD risk, and all-cause mortality [52,53]. This relationship has been difficult to study because dietary sodium intake is not easily measured by standard dietary assessment methods. Salt added at the table and during cooking is difficult to quantify, and processed foods vary widely in sodium content. Most often, 24-hour urine collections are used to assess daily sodium intake. Under stable conditions (e.g., adequate health, hydration, no excessive sweating), 90–95% of dietary sodium is excreted in the urine [54]. Nonetheless, wide variations in day-to-day sodium excretion within individuals will weaken the correlation of single 24-hour urinary sodium levels with BP. This weakness may be minimized by collecting multiple samples in individuals or by increasing sample size in group analyses. Neither solution may be feasible; however, it is possible to employ statistical methods to correct for this source of error by using data from repeated collections in a subset of the study population [55,56]. Improperly collected urine samples and varying geographic conditions (e.g., climate) among populations [9] can introduce additional error. Despite these methodologic limitations, a relationship between dietary sodium intake and BP is well established and supported by many studies.

1 Observational Studies

A direct relationship between sodium intake and BP is repeatedly observed across populations [57–61]. For example, the INTERSALT study measured the relationship between 24-hour urinary sodium excretion and BP in 10,079 men and women from 52 centers around the world [33].

After appropriate adjustments, including correction for measurement error due to use of single 24-hour urine collections, a 100 mmol/day (2300 mg sodium) increase in urinary sodium was associated with an increase in SBP of 3–6 mmHg and DBP of 0–3 mmHg [62]. The INTERSALT investigators also reported a significant relationship between sodium intake and the slope of BP increase with age across populations [33], suggesting a role for sodium in age-related BP increase.

2 Interventional Studies

The early observation of a large BP reduction in patients with severe hypertension consuming the Kempner rice diet [63] is often attributed to its low sodium content (7 mmol or 150 mg/day), though the diet was also rich in fruit, low in fat and protein, and supplemented with vitamins. The many trials of sodium reduction conducted since then have demonstrated a BP-lowering effect of reducing sodium intake, but have differed with regard to the magnitude of effect. Metaanalyses of numerous clinical trials of sodium reduction demonstrate that reduction to currently recommended levels (2300 mg/day) lowers BP by approximately 3–6/1–3 mmHg in hypertensives and 1–2/1 mmHg in nonhypertensives [64–66].

Given the difficulties in measuring sodium intake, as noted previously, perhaps the most compelling trial data

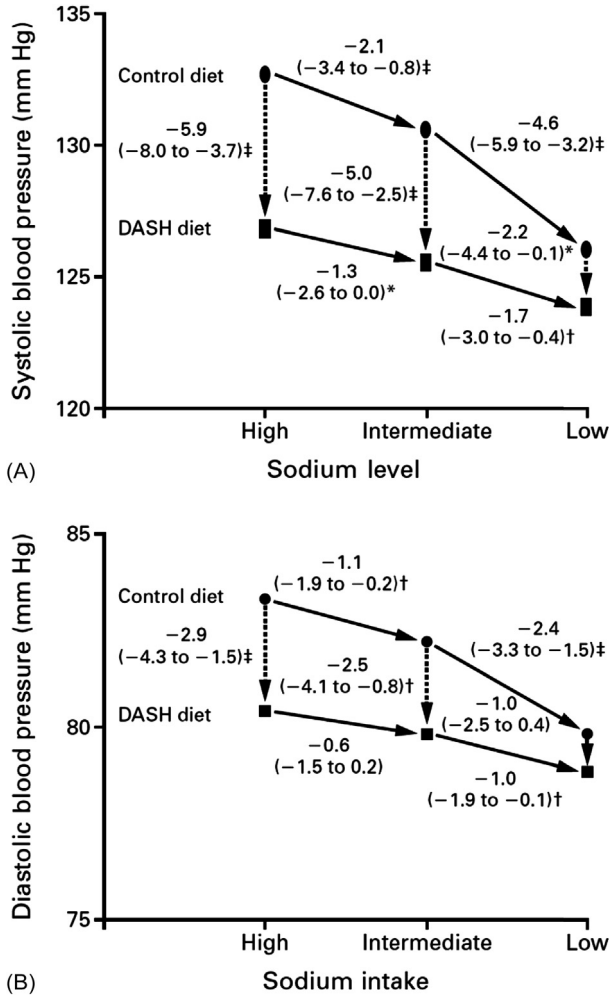

FIGURE 28.2 The effect on Systolic Blood Pressure (SBP) (Panel A) and Diastolic Blood Pressure (DBP) (Panel B) of Reduced Sodium Intake and the DASH Diet. *Source: F.M. Sacks, L.P. Svetkey, W.M. Vollmer, et al., Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. New Eng. J. Med. 344(1) (2001) 3–10 [25]. *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.*

come from the DASH-sodium trial, a controlled feeding study in which nutrient content was precisely determined in a metabolic kitchen and all foods and beverages were provided to study participants. DASH-sodium trial demonstrated that the effect of sodium intake on BP is markedly influenced by dietary pattern: in the context of a typical American diet, reducing sodium intake from an average of 3500 (High) to 2400 mg/day (Intermediate) led to an average reduction in BP of 2.1/0.6 mmHg, and reduction from 3500 to 1500 mg (Low) reduced BP by an average of 6.1/3.5 mmHg (Fig. 28.2) [25]. But when participants were eating the DASH dietary pattern (see below), reducing sodium intake had about half that effect. As with the fruits and vegetables diet in the DASH study, effects of reducing sodium were more pronounced in those with hypertension compared to prehypertension and in blacks compared to whites [25].

Although reducing sodium lowered BP more while eating a relatively unhealthy diet, the combination of DASH and reduced sodium had a larger BP-lowering effect than either dietary change alone, leading to current recommendations to implement both changes [67].

3 Mechanism

A high sodium intake may affect BP via sodium retention and/or via effects on endothelial health [68,69]. It is clear that there are genetic influences on the BP response to sodium intake [70], and other dietary components may interact with sodium's effect on BP [71,72].

4 Summary

The preponderance of evidence supports the 2015–2020 Dietary Guidelines for Americans recommendation that individuals 14 years and older consume sodium less than 2300 mg/day. The guidelines also state that those younger than 14 years follow the age and gender-appropriate upper limits (UL) [50].

In addition, the AHA [73] recommends that those with hypertension or prehypertension attempt to further reduce sodium intake to 1500 mg/day to receive even greater benefit on BP. This recommendation is consistent with the recommendations of the World Health Organization (WHO) [74] and the Institute of Medicine (IOM) [75]. However, some have expressed concern that trying to achieve intake of 1500 mg/day or less is not feasible for most people with the current food supply as it would require marked change in dietary habits and purchasing expensive no-salt products (e.g., bread, cheese).

In any case, food labels and modest public and patient education can facilitate achieving the goal of sodium intake of 2300 mg/day or less, with expected beneficial effects on BP.

C Calcium

Although a modest BP-lowering effect of calcium is noted in some observational studies, results from intervention trials have been inconsistent. Studies showing the greatest effect have tended to use dietary sources of calcium (e.g., dairy products), in which several potentially confounding dietary factors also change [24]. In addition, the BP-lowering effect of calcium may be greater among those with a low habitual intake of calcium.

1 Observational Studies

The higher calcium and magnesium content of "hard" water, and its inverse relation to cardiovascular mortality [76] initially sparked epidemiologic investigation into the relation of both minerals to BP. Cutler and Brittain [77] reviewed 25 observational studies and showed only modest associations between calcium and BP. Because most studies used 24-hour recall methods to assess diet, random day-to-day variation may have obscured any relation with BP [77]. It is also worth noting that some low-BP populations have minimal calcium intakes, [14] more counterevidence to the link between calcium and BP. Cappuccio and others [78] later conducted a meta-analysis of 23 observational studies, and found negligible associations for calcium with both SBP and DBP, though a reanalysis of this data resulted in a somewhat stronger inverse association [22]. A cross-sectional analysis of the NHANES III data showed that increasing calcium intake is significantly associated with a lower rate of age-related increase in SBP and pulse pressure [79]. Other cross-sectional or longitudinal studies also have shown a significant and inverse association between calcium intake [34,80–82] or dairy intake [83–86] and BP.

2 Interventional Studies

In 1924 Addison and Clark [87] recorded the BP response to repeated administration and discontinuation of oral calcium supplements in a convenience sample of hospital outpatients and inpatients. They noted that BP decreased after 2 weeks of calcium supplementation and immediately rose with calcium discontinuation. It was not until much later in the century that the calcium hypothesis was tested in a more methodologically rigid fashion in a number of intervention studies.

Several meta-analyses of calcium-intervention trials have been published since 1989 [88–93], all showing only a slight or negligible BP reduction, primarily of SBP, with calcium supplementation of about 1000 mg. Intervention studies using calcium from food sources have sometimes [94], but not always [95], been more effective, though these studies also involve changes in several other nutrients.

A randomized controlled trial (RCT) in 732 postmenopausal women showed that calcium supplementation of 1 g/day significantly reduced SBP by 1–2 mmHg at 6 months; however, this effect disappeared at 30 months despite continual calcium supplementation [96]. Two other small randomized trials did not find any significant effect of calcium supplementation on BP [97,98]. However, supplementing 1000 mg calcium plus 50000IU Vitamin D3/day for 12 weeks significantly improved BP among 60 pregnant women [99]. A daily supplement of three servings of dairy products significantly reduced SBP among men with high normal or hypertension but not in women [100]. An interaction between calcium and other minerals in their effect on BP has been observed [101] such that calcium supplementation may prevent a salt-induced rise in BP in susceptible individuals. Inability to either control for sodium intake or stratify by level of sodium intake in most meta-analyses may obscure a relationship.

Despite weak evidence for a role of calcium in BP, the potential impact of calcium on cardiovascular health has been examined with inconsistent findings [102,103]. A meta-analysis of 11 prospective studies involving 757,304 participants concluded that there is a nonlinear association between dietary calcium intake and risk of CVD mortality [104]. Intakes that were lower and higher than about 800 mg/day were gradually associated with a higher risk of CVD mortality. Further, the risk of all-cause mortality did not decrease at intakes above 900 mg/day. Thus the role of calcium in BP and CVD health remains to be examined and clarified.

3 Mechanism

Potential mechanisms by which calcium may affect BP include effects on plasma renin activity, endothelial function, or the production of nitric oxide (Fig. 28.1).

4 Summary

Overall it is advisable for the public to consume adequate dietary calcium, preferably from food sources, for the benefit of bone health. The Dietary Reference Intakes (DRI) for calcium suggests an intake of 1000 mg/day for all adults and 1200 mg/day for women 51 years and older and men 71 years and older. This level of intake is similar to that tested in the DASH dietary pattern and may also promote BP health.

D Magnesium

In animal models magnesium deficiency has been shown repeatedly to be associated with hypertension [105–107]. In humans, magnesium deficiency, although recognized rarely, is seen in severe malnutrition, in chronic alcoholism, and in association with malabsorption [108].

1 Observational Studies

As with calcium one of the early suggestions for a role of magnesium in hypertension came from reports that water hardness (increased calcium and magnesium) was associated with lower cardiovascular mortality [76]. This finding was corroborated by Yang and Chin [109]. A more recent examination of dietary and plasma magnesium and risk of CHD was conducted among 86,323 women in the Nurses' Health Study [110]. The examination found a

significant inverse association between magnesium intake and risk of fatal CHD and this association appeared to be mediated partially by hypertension. Several cross-sectional [111,112] and prospective observational analyses [113,114] have found higher magnesium diets to be associated with lower BP. However, interpretation of these results is limited by the fact that a high magnesium diet tends to be high in other beneficial dietary factors as well. In a prospective study magnesium intake was found to be inversely associated with risk for hypertension after adjustment for known risk factors among 28,349 participants of the Women's Health Study [115]. Low serum magnesium (hypomagnesium) was significantly associated with the prevalence of prehypertension among 4,272 Mexican adults [116]. In addition, among 5,511 participants in the PREVEND trial, urinary magnesium excretion was inversely associated with risk of hypertension [117]. Dietary intake of magnesium was also found to be inversely associated with SBP in this study and with both SBP and DBP in another cross-sectional study [112].

2 Interventional Studies

Many intervention trials of magnesium supplementation have been conducted [27,39,118–128] and some have shown a beneficial effect on BP [118,119,124,125,127]. Patients were magnesium depleted (due to diuretic treatment) in two of the studies [118,119], and in one study an effect was only seen in those with a low baseline intake of dietary magnesium [128]. Kawano et al. [126] found the greatest BP reduction with magnesium supplementation in older men on antihypertensive medications. More than half of these studies, however, found no BP-lowering effect with magnesium supplementation. In a meta-analysis of 12 randomized trials, magnesium supplementation significantly reduced DBP only (by 2.2 mmHg) but had no significant effect on SBP [128].

Epsom salt is magnesium sulfate that has been suggested to benefit BP in some media. There has been very little research conducted to examine its impact on BP. The potential benefit of Epsom salt on BP may be related to its magnesium content. Individuals are advised to consume adequate magnesium from natural food sources instead of taking Epsom salt.

Overall, the quality of the included trials was poor, results varied, and thus the evidence was weak. In another meta-analysis of nine randomized trials in participants with diabetes, a median level of 360 mg/day magnesium supplementation did not have any significant effect on BP [129].

3 Mechanism

Adequate magnesium is required for the Na/K-ATPase pump, which regulates intracellular calcium—one of the critical determinants of vascular smooth muscle contraction [108] (Fig. 28.1).

4 Summary

Despite the potential role for magnesium in BP control, evidence from intervention trials to date has not been supportive. However, individuals are encouraged to consume adequate amount of magnesium from natural food sources as recommended by adopting the DASH eating pattern (see Section V-A).

E Vitamin C

Many studies have examined the association between vitamin C intake and BP regulation and findings are inconsistent [130].

1 Observational Studies

Earlier observational studies have shown an association between either vitamin C intake [36] or plasma ascorbic acid [131] and BP. A recent prospective cohort study of 2,884 adults showed that dietary intake of vitamin C and several food groups high in vitamin C were inversely related to hypertension whereas supplemental vitamin C was not [132]. In addition, this study showed that plasma ascorbic acid independently predicts hypertension.

2 Interventional Studies

Most interventional studies employed vitamin C supplements and the findings have not been as consistent as that from observational studies. In a meta-analysis of 29 clinical trials with 10–120 participants, the median dose of vitamin C supplement was 500 mg/day and the median duration was 8 weeks [133]. The pooled changes in SBP and DBP were −3.84 mmHg (95% CI: −5.29, −2.38 mmHg; $p < .01$) and −1.48 mmHg (95%CI: −2.86, −0.10 mmHg; $p = .04$), respectively. Corresponding reductions were greater among those with hypertension (−4.85/−1.67 mmHg, $p < .01$/$p = .17$). In a study of 32 untreated hypertensive patients and 20 normotensive subjects, acute infusion of vitamin C (3 g intravenously in 5 minutes) significantly reduced BP in hypertensive patients but not in normotensive subjects [134]. Intravenous vitamin C alone (>30–100 g) reduced the mean BP up to 8–9 mmHg in prehypertensive patients [135]. Thus, short-term or acute effect of vitamin C supplementation was consistently observed, however, BP was not changed after 5 years supplementation of either 50 or 500 mg/day among 439 Japanese participants with atrophic gastritis [136].

3 Mechanism

Potential mechanism for the role of vitamin C on BP regulation mainly relates to its antioxidant function. Supplementation of vitamin C may improve arterial stiffness and endothelial function, which subsequently may reduce BP [137]. Vitamin C supplementation (2 g orally) may also impact BP through its effect on endothelium-dependent vasodilatation as observed in the radial circulation of patients with coronary artery disease [138].

4 Summary

Overall evidence supports the role of vitamin C on BP regulation; however, long-term effect is less clear. Individuals are advised to consume a dietary pattern rich in fruits and vegetables (8−10 combined servings of fruits and vegetables per day) to ensure adequate and sufficient intake of vitamin C.

III MACRONUTRIENTS

Studies of macronutrients and BP are subject to the same design limitations as studies of micronutrients described earlier. For example, a study designed to examine the effect of the amount of fat intake on BP under isocaloric conditions inevitably will alter the amount of protein and/or carbohydrate, as well as other nutrients consumed. As a result it may be difficult to attribute changes in BP to change in fat intake alone. In addition, BP may be influenced by not only the quantity of macronutrient consumed, but also the type of macronutrient and the food source that it is derived. Both aspects can affect BP independently, but they are not always distinguishable in research designs.

A Protein

1 Observational Studies

Observational studies continue to show an inverse relationship between dietary protein intake and BP [139−142], particularly with plant protein. Cross-sectional studies conducted in rural Japanese and Chinese populations found only an inverse relationship between animal protein and BP [143,144]. In contrast, cohort studies conducted in the United States have shown an inverse association between SBP and baseline intakes of plant protein but not animal protein [145,146]. Thus, there may be differential effects of animal and plant protein on BP, but the exact relationship is not clear.

2 Interventional Studies

It should be noted that many intervention trials employ different types or amounts of protein, and the delivery mechanism may differ as well, all of which contribute to variation in results. Using data from the PREMIER clinical trial [29], we found that dietary plant protein intake was inversely associated with both SBP ($p = .0009$) and DBP ($p = .0126$) in cross-sectional analyses at 6-month follow-up [147]. An increase in plant protein intake was marginally associated with a reduction of both SBP ($p = .052$) and DBP ($p = .082$) from baseline to month 6, independent of change in body weight. Furthermore, among those with prehypertension, increased intake of plant protein was significantly associated with a lower risk of developing hypertension at 6 months. Animal protein was not associated with BP or change in BP in PREMIER. In contrast, Hodgson et al. [148] demonstrated in a small trial that replacing carbohydrate with lean red meat while keeping total calorie and fat intake constant reduced SBP but not DBP. Although the reduction in SBP was statistically significant, the magnitude within the active intervention participants was quite small (−1.9 mmHg using clinic BP and −0.6 mmHg using ambulatory 24-hour BP).

A meta-analysis of 15 RCTs showed no significant difference between high and low intakes of protein on BP [149]. However, other intervention studies have shown a positive impact on BP of increasing protein intake or protein supplement, particularly from plant sources [150−152]. In a 6-week randomized three-phase crossover trial in subjects diagnosed with hypertension, substitution of protein for carbohydrate led to a decrease in SBP of 3.5 mmHg [26].

Most trials use protein supplements that include soy, vegetable, or animal protein and do not control for other nutrients.

The results of numerous RCTs that supplemented soy protein have been mixed. In two randomized double-blind controlled trials, He et al. demonstrated a significant decrease in SBP and/or DBP with soybean protein supplements or with milk protein supplement [153,154]. Further Rivas et al. [155] demonstrated that soy milk supplements significantly reduced SBP by 18.4 mmHg in a 3-month randomized trial. In contrast three RCTs [156−158] showed no difference in SBP or DBP between the soy protein-supplemented group and the placebo group. A meta-analysis of 27 RCTs showed that soy protein supplement was associated with BP reduction and the association was greater among hypertensives [159].

It should be noted that some studies used milk supplements, which provide not only protein but also other nutrients that may affect BP. Other studies that used soy products (e.g., soy nuts) contain other nutrients such as isoflavones that may also impact on BP. Thus, the reported positive impact on BP cannot be attributed to protein alone.

3 Mechanism

Although the exact mechanism(s) linking amount or type of protein to BP is still unclear, several mechanisms have been suggested. First, an increase in protein intake may induce increases in renal plasma flow, renal size, glomerular filtration rate, and sodium excretion [160,161]. Second, the amino acid arginine, a precursor for the vasodilator nitric oxide, may act as a vasodilator and contribute to BP lowering [162]. One possibility that soy protein was hypothesized to reduce BP may be related to its rich content in arginine. Other amino acids including glutamic acid, leucine, and tyrosine may also impact arterial stiffness and benefit BP control [163]. On the contrary, diets high in amino acids rich in animal sources such as glycine may adversely affect BP [164].

4 Summary

Despite controversial evidence that protein restriction may preserve kidney function, especially in those with existing kidney disease [165], the overall effect of protein on BP appears to be salutary. Considering the evidence available, it is advisable to ensure an adequate protein intake on the basis of a healthy eating pattern, such as the DASH eating pattern that may likely benefit BP. Further selecting protein from mainly plant sources may benefit BP control and overall cardiovascular health.

B Dietary Fat

Numerous studies have investigated the relationship between dietary fat and BP. However, because of differences in study design, lack of adequate sample size, and other design limitations, the issue remains controversial. Both the absolute total intake of dietary fat and the relative fatty acid composition may independently relate to BP control.

1 Observational Studies

Most observational studies have not found an association between total fat intake and BP [30,166–168]. However, type of fat intake has been found to be associated with BP in two large studies [166–168] but not others [30,169] that showed a positive relationship between saturated fatty acids and BP. In addition, Hajjar and Kotchen [170] examined the NHANES III data and reported that the southern region of the United States, which consumed the highest amounts of monounsaturated (MUFA) and polyunsaturated fats (PUFA), also had the highest SBP and DBP compared to other regions. Conversely the INTERMAP study found that MUFA intake was significantly and inversely associated with DBP among 4,680 participants from four countries [171]. A significant and inverse association was also found between oleic acid (a MUFA) intake and BP. In a cross-sectional study of 2,447 Chinese adults, serum fatty acids characterized by high proportion of saturated fats was associated with higher SBP while high proportion of PUFA was associated with lower DBP [172]. Similarly a meta-analysis of 8 prospective cohort studies with 56,204 adults found that circulating, but not dietary, long chain m-3 PUFA, especially docosahexaenoic acid (DHA), was significantly and inversely associated with incidence of elevated BP [173]. Thus these observational findings support a beneficial role of PUFA and MUFA and an adverse role of saturated fats on BP.

2 Interventional Studies

Many studies have examined the impact of the amount and/or type of fat on BP. However, as discussed previously in this chapter, the effect of any change in total fat intake on BP is often confounded by changes in other dietary factors or use of fat supplements. Thus the BP responses may not be attributed solely to the change in fat intake. In a crossover randomized study, consumption of a single high-fat meal (42 g) was found to increase both SBP and DBP significantly more than a low-fat meal did (1 g) [174]. Ferrara et al. [175] investigated the effect of MUFA versus PUFA on BP in a 6-month double-blind randomized crossover study. The MUFA diet reduced SBP and DBP significantly more than the PUFA diet. Later, Appel et al. [26] reported that substituting MUFA for saturated fats significantly reduced SBP by 2.9 mmHg in a group of 191 subjects with prehypertension or stage I hypertension. This study finding suggests that a high-fat intake (37% energy) mainly provided as MUFA, in combination with other beneficial dietary factors, can effectively lower BP.

Many short-term intervention trials have been undertaken to determine whether supplementation of either fish or fish oil lowers BP. Because of variations in research design, participant criteria, dosage and type of supplements, and length of intervention, the results have been inconsistent. A meta-analysis of RCTs (36 of which included fish oil) explored the impact of fish oil intake on hypertension prevalence in five populations (Finland, Italy, the Netherlands, the United Kingdom, and the United States) [176]. Pooled metaregression of data from these trials showed that a 4.1 g supplement of fish oil was associated with a 2.1/1.6-mmHg decrease in SBP/DBP. Further a RCT testing 5 oil supplements varying in amounts of *n*-3, *n*-6, and *n*-9 fatty acids among 130 adults found that a high oleic acid, canola oil, with DHA significantly reduced SBP after 4 weeks [177]. Other oil blends did not reach significant BP impact. An olive oil supplement also was found to reduce SBP after 3 months among 41 older adults [178]. When fish intake was examined with its potential impact on BP, one study found a

beneficial association with BP while the other found no effect [179,180].

3 Mechanism

Research has suggested that increased intake of total fat and saturated fats may impair endothelial function, which may subsequently affect BP (Fig. 28.1). Conversely diets rich in omega-3 fatty acids may improve endothelial function and therefore lower BP, the exact mechanism is unclear. How fat intake and various fatty acids affect BP and whether an interaction exists among these factors awaits future research.

4 Summary

Despite the promising benefit of MUFA, PUFA, or fish oil on BP, long-term studies are required to confirm the benefit. Until such information is available, it is more advisable to encourage greater fish consumption as part of a healthy diet than taking fish oil supplementation. Further the current dietary guidelines for Americans in minimizing saturated fats and replacing with MUFA and PUFA should be encouraged [50].

C Carbohydrates

Very few observational and interventional studies have been designed specifically to investigate the impact of the quantity or type of carbohydrate intake on BP. Nevertheless studies of the relationship between fat intake and BP often alter intakes of both fat and carbohydrate while keeping protein intake constant. Thus interpretation of the effect of dietary fat on BP is potentially confounded by the effects from dietary carbohydrate. Nonetheless these studies provide some observation on the potential impact of carbohydrate intake on BP.

1 Observational Studies

As mentioned earlier examination of carbohydrate intake is often confounded by other nutrients or dietary factors. Using the NHANES III data quintile of carbohydrate intake was not associated with the risk for increased SBP ($\geq$140 mmHg) after adjustment for potential confounders and total sugar intake [181]. In fact, three cross-sectional studies, using NHANES 1999–2004 and 2003–2006 data, showed that sugary drinks or fructose consumption was positively associated with a higher BP [182–184]. Yet a cross-sectional study of 814 youth did not find a significant association between fructose intake and BP [185]. In a secondary analysis of the PREMIER clinical trial data, reduction of sugary drink was also found to be associated with a decreased SBP/DBP [186]. Similar finding were observed in one study of 320 children [187] but not another study of 2286 youth with type 1 diabetes [188]. Together these findings suggest a negative impact of simple carbohydrate intake on BP. Further a high intake of whole grains has been shown to be associated with a reduction of hypertension risk in the Women's Health Study [189] and the Health Professionals Follow-up study [190].

2 Interventional Studies

Manipulation of the amount of carbohydrate in interventional studies is often associated with changing intake of other nutrients if total energy is to be kept constant. In the context of whole dietary pattern change, various levels of carbohydrate intakes in combination with other dietary changes have been associated with various findings in BP lowering [24,26,181,191–193].

Some human studies have examined the impact of the type of carbohydrate on BP, but the studies have been limited in number and yielded inconsistent results. In one study [194] SBP rose significantly 1 hour after ingestion of a sucrose or glucose solution, but not after ingestion of fructose. In contrast, a fructose drink, but not glucose, was found to significantly increase SBP/DBP within 2 hours in another study [195]. However, in an earlier study of patients with coronary artery disease, both SBP and DBP decreased after 4-days of a sucrose and fructose load at 4 g/kg/day but not glucose load [196]. In another study that was designed to examine the metabolic effect of sucrose in a group of overweight women, BP was not changed over 6 weeks after consuming a hypocaloric diet with sucrose as the main source of carbohydrate [197]. A more recent RCT also showed that consuming sugar-reduced foods for 8 weeks had no impact on BP as compared to consuming regular sugar foods [198]. Similarly consuming a milk drink sweetened with either sucrose, high fructose corn syrup, fructose, or glucose daily for 10 weeks did not change BP or uric acid level among 267 adults [199].

In a meta-analysis comparing high-carbohydrate to high-monounsaturated fat diets, the high-carbohydrate diet resulted in slightly higher SBP/DBP (2.6/1.8 mmHg) while energy intake and weight were kept constant [200]. Thus the impact of the amount or type of carbohydrate on BP is still inconclusive.

3 Mechanism

Although not well understood, carbohydrate may contribute to the development of essential hypertension through its glycemic effect. Kopp [201] suggests that consumption of a high-glycemic-index diet may create a chronic state of postprandial hyperinsulinemia, sympathetic nervous system overactivity, and vascular remodeling of renal vessels leading to chronic activation of the renin–angiotensin–aldosterone system and

development of essential hypertension. Although logical, the relationship of a high-carbohydrate diet to high BP has not been consistent in all studies.

4 Summary

It is unclear if the amount of carbohydrate alone or the type of carbohydrate affect BP. Future research designed specifically to examine these questions is needed to help clarify the roles of carbohydrate in BP control. Nevertheless, evidence is strong that consuming whole grains may benefit BP and overall cardiovascular health and should be encouraged as the main type of carbohydrate source. Limiting simple carbohydrate such as sugar or sugar-sweetened foods may benefit BP control either directly or indirectly via weight control.

D Fiber

The impact of both the amount and type of fiber on BP has been examined by many studies.

1 Observational Studies

Both cross-sectional [146,202–204] and prospective analyses [205–207] have consistently demonstrated inverse associations between fiber intake and BP but have also noted a high correlation of fiber with other nutrients that can affect BP in a salutary manner. Intake of either total fiber or insoluble fiber have been associated with lower BP. Further dietary fiber intake has also been associated with a lower risk for stroke in a prospective study with 69,677 adults [207] and with a healthier profile of metabolic syndrome markers [204,208].

2 Interventional Studies

Many interventional studies have examined the effect of fiber on BP with varying modes of increasing fiber. Earlier studies suggest that an average supplementation of 14 g fiber reduces SBP/DBP by about 1.6/2.0 mmHg, respectively [209]. Similarly a meta-analysis of 25 RCTs between 1966 and 2003 demonstrates that fiber supplementation (average 11.5 g/day) leads to a reduction in SBP/DBP of 1.13/1.26 mmHg [210]. More recently another meta-analysis analyzed 18 RCTs lasting at least 6 weeks each from 1990 to 2013 and the intervention used either fiber isolate or fiber-rich foods. This analysis showed that all fiber types combined was significantly associated with lower DBP but not with SBP with each 1 g increase in total fiber associated with a 0.11 mmHg reduction in DBP [211]. However, a higher intake of a particular fiber type, β-glucan, was significantly associated with reduction in both SBP and DBP.

3 Mechanism

Potential mechanisms through which fiber may benefit BP include impact on postprandial glucose and insulin response, reduction of insulin levels [209], LDL-cholesterol lowering action and/or endothelial function [212]. This is supported by the observation that hyperinsulinemia is often associated with obesity, impaired glucose tolerance, and hypertension.

4 Summary

Thus, the previous evidence suggests a consistent and small beneficial effect of fiber on BP. Individuals should be encouraged to increase fiber intake to the current recommended level not only for BP control but possibly for other benefits to cardiovascular health. Such recommendations should be achieved by increasing fruits, vegetables, and whole grains based on the foundation of a healthy eating pattern rather than using a supplement.

IV OTHER FOODS AND DIETARY FACTORS

Many food items and dietary factors have been suggested to affect BP regulation with varying levels of evidence.

A Alcohol

1 Observational Studies

Alcohol consumption is directly associated with BP and with prevalence of hypertension in observational studies [213]. Earlier studies showed that men who consume three or more drinks per day [214], and women who consume two or more drinks per day [215] may be at higher risk, but levels below this are not associated with increased risk. In a cohort study of 8334 participants from the Atherosclerosis Risk in Communities (ARIC) study [216], participants were free of hypertension at baseline and were followed for 6 years. Risk of incident hypertension was increased by 20% in those who consumed greater than or equal to 210 g alcohol (78 oz. wine, 191 oz. beer, or 21 oz. liquor) per week as compared to those who did not consume alcohol.

Similarly in a prospective study of 28,848 women from the Women's Health Study and 13,455 men from the Physician' Health study free of hypertension at baseline, alcohol intake was positively and significantly associated with the risk of hypertension in men [217]. Whereas the association between alcohol intake and hypertension was J-shaped in women. The threshold above which alcohol became deleterious for hypertension risk occurred at ≥ 4 drinks/day in women versus ≥ 1 drink/day in men. In a meta-analysis of 16 prospective studies (33,904 men and 193,752 women), increased risk

of hypertension was associated with heavy alcohol consumption of 31−40 g/day (RR, 1.77; 95% CI: 1.39−2.26; $p < .001$) and >50 g/day (RR, 1.61; 95% CI, 1.38−1.87; $p < .001$) [218]. However, significantly increased risk was observed in women when alcohol consumption was 31−40 g/day (RR, 1.19; 95% CI: 1.07−1.32; $p < .002$) and a protective effect was observed at <10 g/day (RR, 0.87; 95% CI: 0.82−0.92; $p < .001$). Thus a J-shaped association between alcohol consumption and hypertension was also observed among the women participants of this study.

Alcohol intake was also shown to have a significant positive relationship with DBP in a linear regression analysis among 1968 adults of the French Nutrition and Health Survey but the association with SBP did not reach statistical significance [219]. Conversely red wine drinkers who consumed ≥1 drink/day had a lower risk of high BP in the cross-sectional PREDIMED study of 3897 adults [220].

Most observational studies seem to support an impact of alcohol on BP, but the relationship between type of alcohol consumed and BP is not consistent. Furthermore chronic habitual intake may be more related to BP than distant moderate to heavy drinking. In one study men who quit drinking alcohol during an 18-year follow-up period experienced less age-related increase in BP than those who did not [221]. A dietary pattern characterized by high drinking was also shown to associate with a higher risk for prehypertension and hypertension [222].

2 Interventional Studies

The relatively few intervention studies of alcohol and BP have tended to be small and of short duration. Cushman et al. [223] reviewed 12 RCTs of alcohol reduction on BP and concluded that a daily reduction of one drink lowers SBP and DBP by approximately 1 mmHg. The Prevention and Treatment of Hypertension Study (PATHS) [224] was designed to evaluate the long-term BP-lowering effect of reducing alcohol consumption in nondependent moderate drinkers (those who consumed more than 3 drinks/day). The goal of intervention was either two or fewer drinks daily or a 50% reduction in intake (whichever was less). After 6 months the intervention group experienced a 1.2/0.7 mmHg greater reduction in BP than the control group (NS), and among hypertensives, this reduction was more modest. In this study the intervention group reduced their intake by 2 alcoholic drinks/day, but the control group also lowered their alcohol intake during intervention, so that the difference in intake between the groups was only 1.3 drinks/day. This small difference between the two groups limits the interpretation of the true BP effect of the intervention group. In addition, perhaps a greater reduction in alcohol consumption is necessary to see a significant effect on BP. However, this level of reduction appears realistic in moderate alcohol drinkers and is similar to the absolute reduction achieved in an earlier study [225].

In another study [226] of mainly heavier drinkers (>5 drinks/day), replacing alcohol with low-alcoholic substitutes resulted in a reduction of approximately 5 drinks/week and a greater reduction in BP (−4.8/3.3 mmHg). Importantly this intervention also reduced body weight of the participants by an average of 2.1 kg, which may explain the larger BP reduction than that observed in the PATHS trial. However, in a meta-analysis [227] of 15 RCTs including a total of 2,234 participants who drank an average of 3−6 drinks/day at baseline, the effect of alcohol reduction on BP was explored. Reduced alcohol consumption, varying from 29% to 100% reduction from baseline, was associated with a significant reduction in mean SBP of −3.31 mmHg (95% CI: −2.52 to −4.10 mmHg) and DBP of −2.04 mmHg (95% CI: −1.49 to −2.58 mmHg), whereas the body weight was minimally changed (mean change: −0.56 kg). There was a dose-response relationship between the mean percentage of alcohol reduction and mean percentage of BP reduction. Effects of intervention were enhanced among those with higher baseline BP.

3 Mechanism

The exact mechanism for an alcohol−BP association is not clear, but possibilities include stimulation of the sympathetic nervous system, inhibition of vascular relaxing substances, calcium or magnesium depletion, and increased intracellular calcium in vascular smooth muscle [213,228,229]. Further the impact of alcohol on BP may also be influenced by a genetic polymorphism in the aldehyde dehydrogenase-2 [230].

4 Summary

Limiting alcohol consumption to the current recommendation of two or fewer drinks per day for men and one or fewer for women is supported by most research evidence and will likely improve BP. There is no need to recommend total abstinence; indeed, light to moderate alcohol consumption has been associated with a reduced risk of multiple cardiovascular outcomes [231] and may be protective against ischemic stroke [232].

B Garlic

Garlic has been shown by many studies to affect BP and most are interventional in nature.

1 Observational Studies

None identified.

2 Interventional Studies

In a meta-analysis of 20 RCTs with 970 participants, garlic reduced SBP/DBP by $5.1 \pm 2.2/2.5 \pm 1.6$ mmHg among all participants and by $8.7 \pm 2.2/6.1 \pm 1.3$ mmHg among those with hypertension [233]. Another umbrella review of eight meta-analyses synthesizing data from both observational and RCTs reported consistent findings of an effect of garlic on BP. Seven of the eight analyses showed a significant reduction in SBP while six of the eight analyses showed a significant reduction in DBP from garlic supplementation [234].

3 Mechanism

Garlic contains many bioactive compounds that may have antioxidative function and allicin may be the most effective compound in lowering reactive oxygen species and stimulating the production of antioxidative substances such as glutathione [235]. Garlic may also affect BP by improving endothelial function [236].

4 Summary

The evidence is consistent that garlic may benefit BP regulation. Various amounts of garlic extract or powder have been used in the interventional studies, ranging from 600 to 2400 mg/day (3.6−13.6 mg allicin) or approximately 2 g raw garlic per day (5−9 mg allicin). Since rare adverse effect has been reported from garlic supplements, this may be an effective and safe strategy to improve BP.

C Flavonoid

Flavonoid includes a group of phytochemicals that may benefit health conditions including CVD and hypertension [237]. Studies examining the impact of flavonoids have employed either supplements or flavonoid-rich foods such as fruits, and vegetables, and thus the association may be attributed to other beneficial factors in addition to flavonoid.

1 Observational Studies

Observational study [238] has shown a lower increase in BP over time when dietary intake of fruits and vegetables was high. Intake of a flavonoid subclass-anthocyanin was found to be inversely related to clinic SBP [239] or risk of hypertension [240]. In a more recent prospective cohort of 40,574 disease-free French women at baseline, intakes of total flavonoid, flavonol, anthocyanins, and proanthocyanidin polymers were found to be significantly and inversely associated with rate of hypertension [240]. Women in the highest quintile had a 9−10% lower rate of hypertension as compared to those in the lowest quintile. Thus these studies support an inverse association between flavonoids and BP.

2 Interventional Studies

Many studies have examined the impact of either flavonoid products or flavonoid-rich foods such as chocolate, tea, fruits, and vegetables on BP. Supplementing 1.4 g/day cocoa extract for 4 weeks reduced postprandial SBP significantly in 24 obese participants [241]. Similarly consuming a cocoa flavonol (450 mg) drink twice daily for a month reduced SBP/DBP significantly (−4.4/3.9 mmHg) [242]. Supplementing 520 mg or 993 mg of cocoa flavanol daily for 8 weeks also reduced BP significantly in 90 elderly individuals [243]. Quercetin appears to be a subclass of flavonoid that has shown more consistent impact on BP [237,244]. However, other studies have not supported an impact of flavonoids or flavonoid-rich foods on BP [245,246]. A meta-analysis of RCTs testing flavonoids or flavonoid-rich foods on endothelial function and BP showed significant effect on BP (−1.46/ − 1.25 mmHg) [247]. In another meta-analysis of 20 RCTs, 30−1080 mg of flavanols (3.6−105 g cocoa products per day) for 2−18 weeks was found to significantly reduce BP by 2.8/2.2 mmHg (both $p < .01$).

Tea is rich in flavonoid and has been suggested to benefit cardiovascular health including BP. Three meta-analyses examined 20, 25, and 13 RCTs separately but included many of the same RCTs in the analyses [248−250]. These analyses concluded that tea consumption reduced BP significantly, ranging from −1.94 to −2.6 mmHg in SBP and from −1.17 to −2.2 mmHg in DBP.

Other flavonoid-rich foods that have been suggested to benefit BP control include beetroot [251,252], dark chocolate [253,254], and grape or grape seed extracts [255,256], however, the evidence is relatively weak due to small sample size. Thus the potential impact of flavonoids or flavonoid-rich foods on BP regulation remains to be clarified and confirmed in properly powered trials.

3 Mechanism

Potential mechanisms of flavonoids' impact on BP regulation are related to its antiinflammatory and antioxidant nature [257]. Flavonoids may benefit BP by improving endothelial function and/or increasing the bioavailability of the vasodilator nitric oxide.

4 Summary

The evidence supports a potential impact of flavonoids or flavonoid-rich foods on BP regulation, however, the impact may be moderate and long-term effect is unclear. Incorporating flavonoid-rich foods like fruits and vegetables is consistent with current recommendation to

prevent or manage hypertension. Other flavonoid-rich foods like tea and cocoa-containing products may also be incorporated into a healthy BP-friendly pattern.

D Vitamin D

Many studies have examined the potential impact of vitamin D on BP or risk for hypertension.

1 Observational Studies

In cross-sectional analyses of data from NHANES III, serum vitamin D was inversely associated with BP [258] and optimal vitamin D status was significantly associated with a decreased age-related increase in SBP by 20% [259]. Data from two large cohort studies also showed that plasma 25(OH)D level was significantly and inversely associated with risk of incident hypertension [260]. Dietary intake of vitamin D, but not vitamin D supplementation, was associated with a lower risk for hypertension [261]. But not all observational studies showed a significant association between either dietary vitamin D intake or serum vitamin D with BP [84,262].

2 Interventional Studies

Many studies have examined the effect of vitamin D supplementation on BP. In a large meta-analysis of 46 RCTs, vitamin D supplement of ≥ 4 weeks did not show an effect on BP [263]. Similarly studies conducted after this meta-analysis did not support an impact of vitamin D supplementation on BP either [264–266].

3 Mechanism

Vitamin D was suggested to be a potent endocrine suppressor of renin biosynthesis and vitamin D deficiency stimulates renin expression [267].

4 Summary

Despite the potential impact of vitamin D on BP, the overall evidence is weak and the strength of impact may be small. Nevertheless vitamin D intake tends to be inadequate in many individuals, thus it is important to encourage regular consumption of vitamin D rich foods such as dairy and certain fish like tuna and salmon.

V DIETARY PATTERNS

The previous sections on micro- and macronutrients highlight extensive confounding due to simultaneous changes in multiple nutrients. It is difficult to study (and change) one component of the diet without affecting others. Thus it may be more appropriate to focus on dietary pattern rather than on individual micro- and macronutrients, and indeed there is extensive evidence that dietary pattern affects BP. For example, vegetarian groups in the United States and abroad have been observed to have lower BP than their nonvegetarian counterparts in many [268,269] but not all studies [270]. The term *vegetarian* comprises several heterogeneous groups [271], but in general, the diet tends to be high in whole grains, beans, vegetables, and sometimes fish, dairy products, eggs, and fruit [272]. Aspects of the vegetarian diet suggested to benefit BP include ample amount of plant foods, a low intake of animal products [269], and a high potassium, magnesium, fiber, (sometimes) calcium [271,272], a high ratio of polyunsaturated to saturated fat, and often a low sodium intake. However, as outlined in previous sections of this chapter, studies on individual nutrients have shown inconsistent results. Explanations for such inconsistencies may include the following: (1) The effect of individual nutrients may be too small to be detected, particularly when trials contain insufficient sample size and thus statistical power; (2) most intervention studies employed supplements of nutrients, which may function differently from nutrients in foods; (3) other dietary factors naturally occurring in foods that are not hypothesized to affect BP may also have an impact on BP; and (4) nutrients occurring in foods simultaneously may exert synergistic effects on BP. Clearly, differences in physical activity, stress, alcohol consumption, and other unmeasured factors may also contribute to a lower BP among vegetarians. But when research participants were counseled to follow the vegetarian diet pattern in intervention studies, significant reductions in BP in both nonhypertensive [273] and mildly hypertensive [272] participants were reported.

Despite the clear BP effect of a vegetarian diet, it is not realistic to expect a wide-scale adoption of this dietary pattern. In addition, a vegetarian diet does not include all dietary factors associated with lower BP. Thus the DASH multicenter trials were designed to test the impact of whole dietary patterns on BP simultaneously while controlling for multiple nutrients, weight, and dietary factors [24,25].

A The Dietary Approaches to Stop Hypertension Dietary Pattern

The original DASH trial [24] was an 11-week randomized controlled feeding trial of 459 individuals with prehypertension or stage I hypertension. Three dietary patterns varying in amounts of fruits, vegetables, dairy products, meats, sweets, nuts, and seeds and thus fats, cholesterol, fiber, calcium, potassium, and magnesium were tested (Table 28.2). In brief, the dietary patterns were (1) the control diet, which mimicked what most

TABLE 28.2 Nutrient Targets for the Three Dietary Patterns Tested in the DASH Trial

Item	Control Diet	Fruits and Vegetables Diet	DASH Diet
Nutrients (2100 kcal/day)			
Fat (% kcal)	37	37	27
Saturated (% kcal)	16	16	6
Monounsaturated (% kcal)	13	13	13
Polyunsaturated (% kcal)	8	8	8
Carbohydrates (% kcal)	48	48	55
Protein (% kcal)	15	15	18
Cholesterol (mg/day)	300	300	150
Fiber (g/day)	9	31	31
Potassium (mg/day)	1700	4700	4700
Magnesium (mg/day)	165	500	500
Calcium (mg/day)	450	450	1240
Sodium (mg/day)	3000	3000	3000
Food groups (servings/day)			
Fruits and juices	1.6	5.2	5.2
Vegetables	2.0	3.3	4.4
Grains	8.2	6.9	7.5
Low-fat dairy	0.1	0.0	2.0
Regular-fat dairy	0.4	0.3	0.7
Nuts, seeds, and legumes	0.0	0.6	0.7
Beef, pork, and ham	1.5	1.8	0.5
Poultry	0.8	0.4	0.6
Fish	0.2	0.3	0.5
Fat, oils, and salad dressing	5.8	5.3	2.5
Snacks and sweets	4.1	1.4	0.7

Americans were consuming at the time the trial was conducted; (2) a fruits and vegetables diet, which contained a macronutrient profile similar to that of the control diet except with a higher amount of fruits and vegetables; and (3) the DASH dietary pattern, which was higher in fruits, vegetables, and low-fat dairy products; lower in total and saturated fats and cholesterol; and rich in fiber, potassium, magnesium, and calcium. Sodium intake, body weight, and alcohol consumption were kept constant throughout the intervention.

The DASH dietary pattern reduced BP by 5.5/3.0 mmHg more than the control group (both SBP and DBP $p < .001$). The fruits and vegetables diet reduced BP by 2.8/1.1 mmHg more than the control diet ($p < .001$ for SBP and $p = .07$ for DBP). The reductions in BP were significant after participants consumed the diets for 2 weeks and were sustained for the following 6 weeks (Fig. 28.3). In addition, BP lowering was similarly effective in men and women and in younger and older persons, and it was particularly effective among African Americans and those who had high BP. These reductions occurred while body weight, sodium intake, alcohol consumption, and exercise patterns remained stable. Of note, sodium intake was not reduced and was identical in all treatment groups (3000 mg/2100 kcal/day). Among the 133 participants with hypertension (SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg), the DASH dietary pattern lowered SBP and DBP by 11.4 and 5.5 mmHg, respectively. These effects in hypertensives are similar to reductions seen with single-drug therapy [274] and more effective

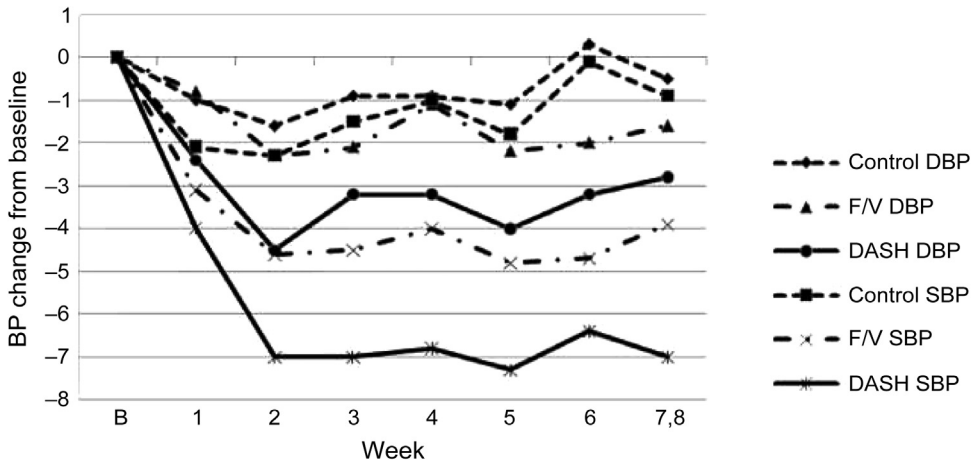

FIGURE 28.3 Mean changes in systolic and diastolic blood pressures from baseline during each intervention week, according to diet, for participants in DASH study.

than most of the other lifestyle modifications for BP reduction (Table 28.3).

Even though the DASH trial was not designed to identify specific nutrient(s) responsible for the BP-lowering effect, data from the fruits and vegetables group support the hypothesis that increasing potassium, magnesium, and dietary fiber intake reduces BP. In addition, by further lowering total and saturated fat and cholesterol, and increasing low-fat dairy products in the DASH dietary pattern, BP reduction was nearly doubled compared to the fruits and vegetables diet. Because whole food items rather than single nutrients were manipulated in this trial, other nutrients that were not controlled for in the study or other beneficial factors as yet unrecognized may also have contributed to the BP responses. Further research is needed to analyze the specific nutrients or factors responsible for the BP effect. Further details on DASH can be found on the following web site: http://www.nhlbi.nih.gov/health/resources/heart/hbp-dash-index.

B Variations of the DASH Dietary Pattern

Even though the DASH trial provided strong evidence of the efficacy of the DASH dietary pattern in reducing BP, this trial alone was not able to test all hypotheses related to dietary pattern and BP. As discussed in the previous sections, research suggests that a high unsaturated fat intake and high-protein intake may benefit BP. Thus the OmniHeart study [26] was designed to further understand the impact on BP of macronutrient variations of the DASH dietary pattern. The dietary patterns tested were (1) the DASH dietary pattern with a slight reduction in total protein (called the carbohydrate diet, with 15% kcal protein instead of 18% tested in the original DASH trial); (2) the DASH dietary pattern with 10% of the carbohydrate energy replaced with unsaturated fats (called the unsaturated fat diet with mainly monounsaturated fats); and (3) the DASH dietary pattern with 10% of the carbohydrate energy replaced with protein (called the protein diet). A total of 164 adults with prehypertension or stage I hypertension were randomized into the three diets in a crossover fashion for 6 weeks each. All three diets lowered BP significantly but the protein and unsaturated fat diets significantly reduced SBP by 1.4 and 1.3 mmHg more than the carbohydrate diet. The further reductions in BP were even greater among those who were hypertensive at baseline.

Thus, these studies have vigorously and consistently proven that whole dietary patterns such as the DASH dietary pattern or the two modified DASH patterns in the OmniHeart study are effective strategies for BP control. As noted earlier DASH in combination with reduced sodium intake lowers BP more than either intervention alone [25]. In addition, both the DASH and OmniHeart studies demonstrate that benefits of adopting a whole dietary approach extend beyond BP to other health indicators such as lipids [26,275]. Many recent observational studies also have consistently shown that consuming healthy dietary patterns such as DASH, the Mediterranean pattern or that recommended by the Dietary Guidelines for Americans is associated with either a lower SBP, DBP, both or risk of hypertension [276–284]. Conversely, dietary patterns associated with more fried food, higher energy density, saturated fat intake and lower fiber intakes have been associated with a higher BP [285,286]. Further, the DASH dietary pattern was shown to enhance the BP response to BP-lowering medication (Losartan) in hypertensive patients [287].

Intervention studies that counseled participants on following the DASH dietary pattern or other healthy patterns also have shown consistent benefit on BP. A meta-analysis of 17 RCTs showed that following the DASH dietary pattern significantly reduced SPB/DBP by 6.74/3.54 mmHg [288]. RCTs testing intervention with

TABLE 28.3 Summary of Evidence Relating Dietary and Lifestyle Factors with BP

Dietary Factors	Strength of Relationship with BP	Direction of Association	Strength of Impact (mmHg)	Potential Mechanism	Recommendation	Those Most Likely to Benefit
Potassium	1A	Inverse	2−11	Vasodilatory; natriuretic	Increase potassium-rich foods to 4700 mg/day	Those consuming high salt diet
Sodium	1A	Direct	2−8	Changes in blood volume and BP regulating hormones	<2300 mg/day (<65−100 mmol/day)	Hypertensives, salt-sensitive individuals, African Americans, elderly, and those consuming typical Western diet
Calcium	2A	Inverse	1−2	Regulation of parathyroid hormone (PTH) and intracellular calcium; natriuretic	2−3 reduced fat dairy products per day	Possibly salt-sensitive individuals
Magnesium	2B	Inverse	1−2	Modification of Na-K/ATPase activity	Maintain adequate magnesium intake	Those depleted in magnesium, e.g., from diuretics
Vitamin C	1B	Inverse	1−3	Endothelial function, arterial stiffness, and vasodilation	Diet rich in fruits and vegetables	
Protein	2A	Inverse	1−2	BP-related amino acid may act as vasodilator	Consume adequate protein, particularly plant sources; limit high fat animal protein	
Fat	1C	Direct for saturated fat, inverse for monounsaturated fats	2−3	Vasodilatory action of prostaglandins (PGE)	Moderate total fat, include more monounsaturated fat, reduce saturated fat	
Carbohydrates	2B	−	2−3		Consume more whole grains and less sugar or sugar-sweetened products	
Fiber	1A	Inverse	1−2	Possible reduction of insulin, lipids, and action on endothelial functions	Increase fiber-rich foods for overall health	Those who have insulin resistance or impaired glucose tolerance
Alcohol	1B	Direct	2−4	Possible action on sympathetic nervous system, inhibition of vascular relaxing substances, calcium, and magnesium depletion	Moderate consumption: ≥2 drinks/men, ≥1 drink/women	Those consuming >2−3 drinks/day
Body weight	1A	Direct	5−20	Lowering blood volume, cardiac output, and insulin resistance, and raising salt sensitivity	Maintain healthy weight; lose weight if overweight or obese	Overweight and obese individuals
Dietary patterns	1A	Depends on factors	5−14	Multiple mechanisms, as above	Follow DASH, Omni-Heart or Mediterranean dietary pattern	All individuals, those at risk for or with hypertension

1A, clear, consistent, and strong randomized controlled trial (RCT); IB, clear RCT but inconsistent results; 1C+, clear, no RCT but strong observational studies; 1C, clear, observational studies; 2A, unclear, consistent RCT—intermediate strength; 2B, unclear, RCT with inconsistent results; 2C, unclear, observational studies.

Mediterranean dietary pattern supplemented with olive oil or nuts, or the Nordic diet also significantly reduced BP [289–291].

Thus a nutritional approach to BP control that involves changes in overall dietary pattern appears to be superior to approaches that manipulate only a small number of nutritional factors. Consuming a healthy dietary pattern such as DASH is effective in lowering BP and may reduce risk for hypertension, CHD, stroke [292], CVD, and heart failure [293]. Further a meta-analysis of 15 cohort studies concluded that following the DASH dietary pattern is associated with a significant reduction in risk of all-cause mortality, CVD, cancer, and type 2 diabetes mellitus [294]. Thus the recommendation to follow DASH may benefit health beyond BP.

VI WEIGHT REDUCTION AND MULTILIFESTYLE MODIFICATION

BP is closely related to body weight and weight loss alone is an effective strategy for BP reduction. Potential mechanisms for the effect of weight loss on BP include suppression of sympathetic nervous system activity, lowered insulin resistance, normalization of BP regulating hormones [295], decreased body sodium stores, decreased blood volume and cardiac output, and reduction of salt sensitivity [296–298].

A Observational Studies

Several observational studies have reported a positive relationship between several indices of body weight or body fatness with BP [299–303]. Fall et al. analyzed data from 30 studies using a Mendelian randomization approach concluded that adiposity has a significant causal effect on BP [304].

B Intervention Studies

Several large-scale clinical trials have been conducted to evaluate different lifestyle modification programs on BP, several of which provide evidence for a BP-lowering effect of weight loss. For example, in the TOHP Phase I [305], weight loss was found to be the most successful intervention in lowering BP compared to sodium reduction, stress management, or nutritional supplements (calcium, magnesium, or potassium). At the 6-month follow-up, men and women in the intervention group lost 6.5 and 3.7 kg, respectively. This level of weight loss was achieved with a fairly rigorous counseling approach aimed at simultaneously reducing energy intake and increasing exercise [306]. At study termination BP fell an average of 2.9/2.3 mmHg overall (after subtracting the BP change in the control group). In this study, some recidivism occurred, and at 18 months, men had maintained a 4.7-kg weight reduction and women, 1.6 kg. After 7 years' follow-up in a subset of study participants, the odds of developing hypertension were reduced by 77% in the weight loss group [307], even though their long-term weight loss was nearly identical to that of the control group (4.9 kg and 4.5 kg, respectively).

As a follow-up to TOHP Phase I, TOHP Phase II [28] was conducted to examine the effects of weight loss and dietary sodium reduction alone, and in combination, on BP in overweight adults. The intervention lasted 3 years and included individual and group counseling meetings focusing on diet, exercise, and social support. The weight loss intervention group ($n = 595$) achieved a mean reduction in weight from baseline to 6 months of 4.4 kg, 2.0 kg at 18 months, and 0.2 kg at 36 months. BP was significantly lower at all time points mentioned and the greater the weight loss, the greater the BP reduction. At 36 months every kilogram of weight loss was associated with a reduction of 0.35/0.45 mmHg in SBP/DBP. Further this weight loss intervention was associated with a 42% reduction in incident hypertension.

This rigorous study not only provides the evidence of BP-lowering effect of weight loss but also supports the possibility of sustaining BP effect after weight recidivism.

In a meta-analysis of 25 RCTs, weight loss of 1 kg was associated with approximately 1 mmHg reduction in both SBP and DBP in individuals with prehypertension [308]. Thus the evidence of an effect of weight loss on BP is strong and consistent. Since weight loss often involves change in dietary pattern, energy restriction, and increased physical activity, implementing multiple simultaneous lifestyle changes to lose weight and improve dietary quality may be an effective strategy for lowering BP.

In the PREMIER clinical trial [29], the effects on BP of two multicomponent lifestyle interventions compared to an advice-only control group were tested for 18 months. The two behavioral interventions were designed to stimulate adoption of what were at the time the well-established lifestyle guidelines for BP control (EST) or the well-established guidelines plus the DASH dietary pattern (EST+DASH). The well-established guidelines included weight loss if overweight (95% of participants), reducing sodium intake to less than 2300 mg/day, increasing physical activity to 180 minutes of moderate activity per week, and alcohol consumption not exceeding 2 drinks/day for men and 1 drink/day for women. A total of 810 individuals were randomized and completed the study. Participants in both intervention groups significantly reduced weight, improved fitness, and lowered sodium intake, and the EST+DASH group also increased fruit, vegetable, and dairy intake. Mean reduction in SBP, net of the control group, was 3.7 mmHg ($p < .001$) in EST group and 4.3 mmHg ($p < .001$) in EST+DASH

group [29]. Each individual lifestyle modification was independently and significantly associated with SBP reduction at 6 and 18 months [309].

Since PREMIER tested the effects of DASH within a combined lifestyle intervention program which included weight loss, sodium reduction, and increased physical activity, the effect of DASH dietary pattern alone among free-living individuals is not clear. The ENCORE study was thus designed to compare the DASH diet alone or combined with a weight management program with usual diet controls among 144 participants with prehypertension or stage I hypertension in a RCT [310]. Clinic BP reduced by 11.2/7.5 mmHg in the DASH alone group and by 16.1/9.9 mmHg in the DASH plus weight management group. Similar patterns were observed for ambulatory BP, pulse wave velocity, baroreflex sensitivity, and left ventricular mass (all $p < .05$). Thus this study confirms the BP-lowering effect of the DASH dietary pattern in a free-living situation and the improvement in vascular, autonomic function, and reduction in left ventricular mass.

Overall these studies demonstrate that individuals can make multiple dietary and lifestyle changes to reduce BP. Although recidivism is often observed, the beneficial effect of lifestyle changes on BP may last beyond the intervention period. Thus it is important to disseminate proven effective intervention strategies and to develop and test new strategies to help individuals maintain these changes long term.

VII CURRENT RECOMMENDATIONS AND IMPLEMENTATION

The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High BP was appointed by the National Heart Lung and Blood Institute to provide evidenced-based clinical guidelines for the prevention and management of hypertension [311]. Current lifestyle guidelines include salt reduction, moderate alcohol consumption, weight loss if overweight, increased potassium intake, regular moderate or vigorous exercise, and following a healthy eating pattern like DASH (Table 28.4). These lifestyle modifications are all recommended as part of the first-line therapy for low-risk individuals, defined as those without diabetes or CVD and with SBP less than 160 mmHg and DBP less than 100 mmHg. They are also recommended in combination with pharmacotherapy for high-risk individuals. These recommendations also apply to individuals with prehypertension in order to prevent the development of high BP.

Although much evidence indicates that diet and lifestyle modification can prevent and treat hypertension, recent reports suggest that about half of hypertensives are not well controlled (SBP/DBP < 140/90 mmHg) [6]. The full potential of diet and lifestyle modification for treatment remains underutilized and poorly realized, both because dietary causes of hypertension have not been completely identified, and because of poor adherence of both the clinicians and the public alike to the established medical and dietary guidelines. Despite data linking overweight and obesity to numerous adverse health outcomes including hypertension, clear guidelines for weight control, and a plethora of weight loss programs, the obesity epidemic continues to grow. Similarly despite clear and long-standing recommendations to reduce sodium intake to 2300 mg/day or less, the average intake in the United States remains around 3400 mg/day. Furthermore according to the 1999–2000 NHANES survey [312,313], Americans are consuming far less than the nationally recommended amounts of fruits, vegetables, fibers, calcium, magnesium, and potassium—all of which are key components of the DASH dietary pattern [314]. During 2007–2010, half of the total US population consumed <1 cup of fruit and <1.5 cups of vegetables daily; 76% and 87% did not meet fruit and vegetable intake recommendations, respectively [315].

Clearly implementing dietary and lifestyle modifications is challenging. Lessons learned from past research indicate that behavioral intervention programs that result in successful behavior change are rooted in social cognitive theory [316] and techniques of behavioral self-management [317]. They are ideally constructed using the transtheoretical or stages-of-change model [318,319] and use motivational enhancement approaches [320,321]. These approaches emphasize the importance of the individual's ability to regulate behavior by setting goals, developing specific behavior change plans, monitoring progress toward the goals, and attaining skills necessary to reach the goals. Self-efficacy (one's confidence in performing a given behavior) and outcome expectancies (one's expectations concerning the outcome of that behavior) are critical mediators of behavior change [321,322]. The transtheoretical model recognizes that behavioral change is a dynamic process of moving through different motivational stages of readiness for change. Different behavioral strategies may need to be emphasized at different times, depending on the individual's stage of change.

In addition, behavioral interventions conducted with small groups can take advantage of the economy of scale and the social support provided by a group of peers. In the PREMIER trial of lifestyle intervention for lowering BP, the behavioral strategies discussed earlier were incorporated into an intervention consisting of frequent group sessions conducted by a trained interventionist. It is also important that any intervention program make efforts to create a culturally appropriate behavioral intervention. Such effort may include but is not limited to (1) having

TABLE 28.4 The DASH Eating Plan

Food Group	Daily Servings	Serving Sizes	Examples and Notes	Significance of Each Food Group to the DASH Eating Plan
Grains[a]	6–8	1 Slice bread 1 oz. dry cereal 1/2 cup cooked rice, pasta, or cereal[b]	Whole wheat bread and rolls, whole wheat pasta, English muffin, pita bread, bagel, cereals, grits, oatmeal, brown rice, unsalted pretzels, and popcorn	Major sources of energy and fiber
Vegetables	4–5	1 cup raw leafy vegetable 1/2 cup cut-up raw or cooked vegetable 1 cup vegetable juice	Broccoli, carrots, collards, green beans, green peas, kale, lima beans, potatoes, spinach, squash, sweet potatoes, tomatoes	Rich source of potassium, magnesium, and fiber
Fruits	4–5	1 medium fruit 1/4 cup dried fruit 1/2 cup fresh, frozen, or canned fruit 1/2 cup fruit juice	Apples, apricots, bananas, dates, grapes, oranges, grapefruit, grapefruit juice, mangoes, melons, peaches, pineapples, raisins, strawberries, tangerines	Important sources of potassium, magnesium, and fiber
Fat-free or low-fat milk and milk products	2–3	1 cup fat-free milk or yogurt 1½ oz. cheese	Fat-free (skim) or low-fat (1%) milk, fat-free low-fat, or reduced-fat cheese, fat-free or low-fat regular or frozen yogurt	Major sources of calcium and protein
Lean meats, poultry, and fish	< 6	1 oz. cooked meats, poultry, or fish 1 egg[c]	Select only lean; trim away visible fats; broil roast, or poach; remove skin from poultry	Rich sources of protein and magnesium
Nuts, seeds, and legumes	4–5 per week	1/3 cup or 1 1/2 oz. nuts 2 tbsp peanut butter 2 tbsp or ½ oz. seeds 1/2 cup cooked dry beans or peas	Almonds, filberts, mixed nuts, peanuts walnuts, sunflower seeds, kidney beans, lentils, split peas	Rich sources of energy, fiber, magnesium potassium, and protein
Fats and oils[d]	2–3	1 tsp soft margarine 1 tsp vegetable oil 1 tbsp mayonnaise 2 tbsp salad dressing	Soft margarine, vegetable oil (e.g., olive, corn, canola, or safflower), low-fat mayonnaise, light salad dressing	The DASH study had 27% of calories as fat, including fat in or added to foods
Sweets and added sugars	< 5 per week	1 tbsp sugar 1 tbsp jelly or jam 1/2 cup sorbet, gelatin 1 cup lemonade	Fruit-flavored gelatin, fruit punch, hard candy, jelly, maple syrup, sorbet and ices, sugar	Sweets should be low in fat

[a] *Whole grains are recommended for most grain servings.*
[b] *Serving sizes vary from 1/2 to 1 1/4 cups, depending on cereal type. Check the product's Nutrition Facts label.*
[c] *Because eggs are high in cholesterol, limit egg yolk intake to no more than 4/week; 1 egg white has the same protein content as 1 oz. of meat.*
[d] *Fat content changes serving counts for fats and oils. For example, 1 tbsp of regular salad dressing equals 1 serving; 1 tbsp of a low-fat dressing equals 1/2 serving; 1 tbsp of a fat-free dressing equals 0 servings.*

intervention encounters take place at a location in the community; (2) employing staff from the same cultural background as participants; (3) selecting foods, music, or examples from within the culture; and (4) involving staff of the same cultural background in program design and in consultation with the potential participants. Despite considerable understanding of the theory and practice of behavior change, additional research is needed to develop effective strategies for sustaining dietary and lifestyle change long term.

VIII SUMMARY

The overall evidence that diet modification can prevent and treat hypertension is strong. The strength of evidence for various factors and respective recommendations are

summarized in Table 28.3. In some cases, the effective intervention strategy and mechanisms involved are still being clarified. Because of various design limitations, inadequate statistical power, and measurement issues, studies of single nutrients, with the exception of sodium, potassium, and fiber, have generally provided inconsistent results. However, when multiple nutrients or dietary factors are combined in a whole dietary strategy, as seen in the DASH and OmniHeart studies, BP is significantly and effectively reduced. Nutrients may have additive or interactive effects when provided together in whole foods. Thus, the current national guideline of lifestyle modification for BP control includes the DASH pattern, sodium reduction, weight loss, increased physical activity, and moderate consumption of alcohol. Concurrent adherence to several recommendations is likely to hold the greatest promise for preventing and treating hypertension and has been shown to be feasible. In addition to addressing unresolved nutritional hypotheses, future research should focus on strategies to motivate and maintain lifestyle changes long term for BP control. At both the population and individual levels, success in dietary and lifestyle intervention relies on multiple levels of support ranging from clinicians to government agencies to private institutes and industries. In particular, partnering with industry to improve the nutritional quality of the food supply, such as reducing sodium, sugar, and saturated and trans fat content of processed foods, and promoting foods and nutrients consistent with the DASH dietary pattern will play a critical role in implementing dietary and lifestyle modifications. Consistent efforts to educate and promote adherence to dietary and lifestyle guidelines by dietetic and other health care professionals are also instrumental to the prevention and management of hypertension.

To learn more about high BP, visit the NHLBI web site at <http://www.nhlbi.nih.gov/health/health-topics/topics/hbp>. DASH is also online at <http://www.nhlbi.nih.gov/health/health-topics/topics/dash>.

Additional handout for public: 'Your guide to lowering your BP with DASH,' U.S. Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute, NIH Publication No. 06–5834. Originally printed December 2006. Revised August 2015, can be accessed at the NHLBI Website: <http://www.nhlbi.nih.gov>.

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Chapter 29

Genetics and Diabetes

Yoriko Heianza[1] **and Lu Qi**[1,2]

[1]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States*

I INTRODUCTION

Type 2 diabetes is a common chronic metabolic disease and its prevalence has been dramatically increasing around the world [1]. In patients with type 2 diabetes, both insulin secretion and insulin sensitivity are impaired, and chronic hyperglycemia is a major symptom [2]. Both genetic and nongenetic factors are involved in the development of type 2 diabetes. Discovery of rare mutations determining monogenic forms of diabetes provides direct evidence for the genetic basis of the disease. To map genetic variations affecting type 2 diabetes, initial methods mainly include family-based linkage analysis and candidate gene association analysis. Since the first genome-wide association study (GWAS) in 2007, more than 90 genome-wide significant susceptibility loci have been identified. Genetic variants affecting metabolic traits related to type 2 diabetes, especially glucose homeostasis (such as fasting glucose, insulin, insulin resistance, HbA1c, etc.), have also been identified, while these loci are not completely overlapping with type 2 diabetes loci. Most of the GWAS-identified genes are not previously considered to play roles in the pathophysiology of type 2 diabetes. Recent studies investigate causal variants and biological mechanisms responsible for the disease, and also explore potential interactions with environmental factors such as diet and lifestyle. In this chapter, we summarize recent advances in these research areas.

II DIAGNOSIS OF DIABETES AND NONGENETIC RISK FACTORS

According to the Seventh Diabetes Atlas from the International Diabetes Federation, it is estimated that approximately 415 million adults had diabetes worldwide in 2015, which is equivalent to 1 in every 11 adults with diabetes [1]. Without concerted action, the number of patients with diabetes could grow by 50% in the next 25 years, meaning that 642 million people would be affected [1]. Diabetes can be classified into the following four general groups: type 1 diabetes, type 2 diabetes, gestational diabetes mellitus (GDM), and specific types of diabetes due to other causes [3]. Type 1 diabetes accounts for 5−10% of diabetes cases, and is due to cellular-mediated autoimmune destruction of the pancreatic β-cells. GDM is diagnosed in pregnant women in their second or third trimester and is not clearly overt diabetes. Specific types of diabetes due to other causes include monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young, MODY), diseases of the exocrine pancreas, and drug- or chemical-induced diabetes. MODY accounts for a small number of patients with diabetes (<5%).

Type 2 diabetes accounts for ∼90−95% of diabetes cases, and is characterized by insulin resistance and impaired insulin secretion. Dysglycemia and insulin resistance can be observed even 10 years before the development of the disease [4,5]. Certain racial/ethnic subgroups (e.g., African-American, Latino, Native American, Asian American, Pacific Islander) are considered to be at high risk of developing diabetes [3]. Overweight or obesity is the most important risk factor for type 2 diabetes [6,7]. Weight gain from early adulthood has been also consistently related to diabetes risk [8,9]. Low birth weight, a marker of prenatal growth retardation, has been also consistently associated with an increased risk of type 2 diabetes [10,11]. Other environmental risk factors, including current smoking habit [12], dietary factors such as high intake of sugar sweetened beverages [13,14] or foods with high glycemic index (GI) and high glycemic load (GL) (GL: calculated by GI × carbohydrate ÷ 100) [15], and less

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intake of coffee [16,17], whole grains [18], dietary fiber [19,20], or magnesium [21], have also been related to increased risk of type 2 diabetes. In epidemiological studies, high levels of physical activity such as brisk walking [22] and habitual muscle training [23,24] have consistently shown associations with reduced risk of diabetes. Recently, short sleeping duration and shift work were found to be related to an increased diabetes risk [25–27].

III HERITABILITY AND MONOGENIC FORMS OF DIABETES

There is extensive evidence for the heritability of type 2 diabetes. Type 2 diabetes is a well-known familial disease, and studies have estimated that risk for type 2 diabetes increased approximately two- to fourfold when one or both parents were affected [28–30]. In population studies, family history of diabetes has a significant, independent, and graded association with the prevalence of diabetes. In twin studies, very high concordance (55–100%) for type 2 diabetes has been reported among monozygotic (MZ) twins. Kaprio et al. examined a nationwide cohort of 13,888 Finnish twin pairs, in which the probandwise and pairwise concordance rates for type 2 diabetes were 34% and 20% among MZ twins and 16% and 9% in dizygotic (DZ) twins, respectively [31]. Poulsen et al. found probandwise concordance was 50% in MZ twins and 37% in DZ twins [32]. In general, twin and familial studies estimate that the heritability of type 2 diabetes ranges from 20% to 70% [32–35].

Identification of rare forms of diabetes, namely monogenic diabetes which results from mutations in a single gene, provides direct evidence to support the role of genetics in development of the disease (Table 29.1).

TABLE 29.1 Genes Involved in Monogenic Diabetes

Disease Region/Gene	Chromosome	Protein/Gene Function
Insulin Secretion Defect		
MODY		
HNF4A	20q13.12	β-Cell transcription factor
GCK	7p15.3-p15.1	Glucose sensor
HNF1A	12q24.2	β-Cell transcription factor
PDX1	13q12.1	β-Cell transcription factor
HNF1B	17q12	β-Cell transcription factor
NEUROD1	2q32	β-Cell transcription factor
Neonatal diabetes		
KCNJ11	11p15.1	β-Cell ATP-sensitive potassium (K-ATP) channel closure
ABCC8	11p15.1	β-Cell K-ATP channel modulator, sulfonylurea response
IDDM2	11p15.5	Insulin production
PTF1A	10p12.2	Pancreatic development
FOXP3	Xp11.23	Immune response control
Wolcott–Rallison syndrome		
EIF2AK3	2p12	Endoplasmic reticulum stress control
Wolfram syndrome		
WFS1	4p16.1	Cellular Ca^{2+} homeostasis
CISD2	4q24	Cellular Ca^{2+} homeostasis
Werner syndrome		
WRN	8p12	DNA-helicase activity
Friedreich's ataxia		
FXN	9q21.11	Mitochondrial iron, transport/respiration

(*Continued*)

TABLE 29.1 (Continued)

Disease Region/Gene	Chromosome	Protein/Gene Function
Hemochromatosis		
HFE	6p21.3	Regulation of iron absorption
Thiamine-responsive anemia		
SLC19A2	1q23.3	Thiamine transporter protein
Insulin Resistance		
Generalized lipodystrophy		
AGPAT2	9q34.3	Phospholipid biosynthesis
BSCL2	11q13	Fat storage
CAV1	7q31.1	Cell growth/differentiation
Partial lipodystrophy		
LMNA	1q22	Nuclear stability, chromatin structure
LMNB2	19p13.3	Nuclear stability, chromatin structure
ZMPSTE24	1p34	LMNA processing
PPARG	3p25	Cellular signaling/fat differentiation
AKT2	19q13.1-q13.2	Cellular signaling
Type A Insulin Resistance		
Rabson–Mendenhall syndrome, Donohue syndrome		
INSR	19p13.3-p13.2	Insulin signaling

Neonatal diabetes mellitus (NDM) and MODY are the two main forms of monogenic diabetes. NDM, which first occurs in newborns and is diagnosed in the first 6 months of life, can either be transient (TNDM) or be permanent (PNDM). Fine-mapping and sequencing technologies have contributed to identifying the mutational spectrum of causal genes and an increased number of novel MODY genes in recent years. Currently, more than 30 different causal genes for NDM or MODY have been identified [36,37] (Fig. 29.1). NDM is most commonly due to a mutation in the *ABCC8* and *KCNJ11* encoding the Kir6.2 subunit of the β-cell K-ATP channel. Since such patients can be successfully managed with sulfonylureas as first-line therapy [38,39], testing for mutations in these genes is meaningful to make the diagnosis and provide effective treatment. MODY usually first occurs in children or adolescents and is characterized by autosomal dominant family history, continued production of endogenous insulin, absence of β-cell autoimmunity, and absence of signs of insulin resistance [40,41]. MODY is most commonly caused by mutations in the *HNF1A* or *GCK* (glucokinase) gene. Mutations in *ABCC8*, *GCK*, *HNF1B*, *INS*, *KCNJ11*, *NEUROD1*, and *PDX1* may lead to both NDM and MODY (Fig. 29.1) [37,42]. In addition, mutations in genes such as *WFS1*, *EIF2AK3*, *CISD2*, *WRN*, *FXN*, *HFE*, and *SLE19A2* also cause monogenic diabetes (Table 29.1) [37]. While most mutations in monogenic diabetes reduce the body's ability to produce insulin, some mutations in genes such as *AGPAT2*, *BSCL2*, *CAV1*, and *LMNA* mainly affect insulin resistance.

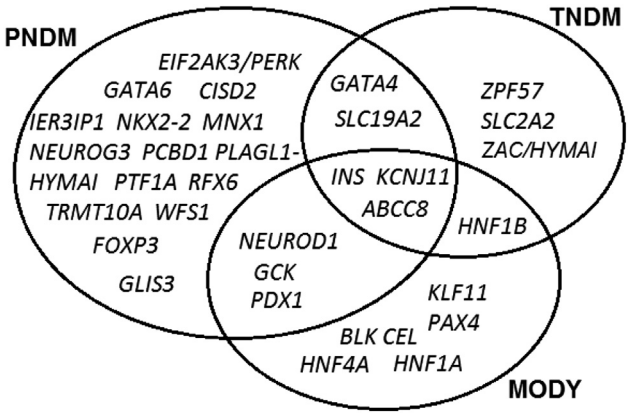

FIGURE 29.1 Monogenic diabetes genes associated with neonatal diabetes mellitus (transient [TNDM] or permanent [PNDM]) and/or MODY.

IV LINKAGE ANALYSIS AND CANDIDATE-GENE-BASED ASSOCIATION STUDIES

The traditional approach for mapping disease genes relies on linkage analysis in affected families followed by progressive fine-mapping of candidate linkage peaks [43]. Linkage is the tendency for genes and other genetic markers to be inherited together because of their position near one another on the same chromosome. Family-based linkage studies are successful in identifying rare variants with a large effect and highly penetrant mutations that underlie Mendelian disorders [44], but these studies are rarely successful in identifying genes that are involved in complex polygenic diseases, such as type 2 diabetes. As a successful example, Hanis conducted the genome-wide linkage scan in Mexican Americans and found a major susceptibility locus on chromosome 2 [45]. Subsequent positional cloning in this region led to the identification of two different haplotypes (121/112) within the *CAPN10* gene conferring higher risk for type 2 diabetes [46]. In 2006, Grant et al. found variants in the *TCF7L2* gene conferring 1.5-fold increased risk of type 2 diabetes in an Icelandic population through fine-mapping a region (chromosome 10q) identified by a previous linkage analysis [47]. The strong association of genetic variants in *TCF7L2* and type 2 diabetes has been consistently replicated in multiple studies, conferring an odds ratio (OR) of 1.4−1.5 per risk allele for type 2 diabetes, which stands out as having by far the biggest effect on diabetes risk [48,49]. Subsequent studies have shown that the originally identified intronic rs7903146 variant is considered to be causative [50,51]. However, the vast majority of linkage studies have not led to the discovery of genes harboring the causal variants for type 2 diabetes.

Since the late 1990s, growing acknowledgment that association studies have considerably greater power than linkage analysis to detect genetic variants with moderate phenotypic effects has instigated the rise of association study as the mainstream approach in the field [52]. In the function-based, candidate-gene association studies, genetic variants in the selected candidate genes are examined based on a hypothesis of pathophysiological mechanisms behind type 2 diabetes. Candidate-gene association studies have discovered several genes such as *PPARG* (peroxisome proliferator-activated receptor gamma) and *KCNJ11* (potassium inwardly-rectifying channel, subfamily J, member 11) as susceptibility loci [53,54], and these findings have been consistently replicated. PPARG is a transcription factor that has a pivotal role in adipocyte differentiation and expression of adipocyte-specific genes. *KCNJ11* gene encodes the Kir6.2 ATP-sensitive potassium channel that regulates insulin secretion by β-cells. Mutations in the *KCNJ11* gene also cause NDM, as discussed above. Both *PPARG* and *KCNJ11* genes encode molecular targets of antidiabetic medications to treat patients with diabetes (thiazolidenediones and sulfonylureas, respectively), and functional relevance makes them compelling candidate genes. Common genetic variants in other genes such as *WFS1* and *TCF2* (MODY5) are also identified using candidate gene association approach [55,56], and these findings have also been replicated in later studies. However, most of the findings from candidate gene association studies are difficult to replicate. Many reasons have been proposed to account for the low reproducibility in the association studies, including the bias introduced by population stratification, lack of power due to small sample size, confounding caused by environmental factors, and heterogeneity in genetic effects [57].

V GWAS OF TYPE 2 DIABETES

The efforts to discover diabetes genes are considerably fueled by GWAS. The new genotyping method comprehensively covers genetic variations on the whole-genome scale, and GWAS is typically designed to study genetic architecture of type 2 diabetes and diabetes-related traits in a hypothesis-free way. Differing from the candidate gene approach, GWAS has unprecedented advantage in uncovering novel genetic contributors and thus lead to insights into the etiology of the disease. GWAS is powerful in identifying common genetic variants (minor allele frequency [MAF] > 0.05) associated with risk of type 2 diabetes, and thus far more than 90 loci have been identified (Table 29.2).

In 2007, the first GWAS on type 2 diabetes identified two novel diabetes loci *SLC30A8* (solute carrier family 30 member 8) and *HHEX* (hematopoietically expressed homeobox) along with confirming the association of *TCF7L2* and *KCNJ11* with type 2 diabetes [58]. The association of *SLC30A8* variant rs13266634 and type 2 diabetes risk was confirmed in subsequent studies across different ethnic groups [59,60]. Other GWAS in 2007 confirmed the associations of *TCF7L2*, *KCNJ11*, *PPARG*, *SLC30A8*, and *HHEX* with type 2 diabetes, and newly discovered *CDKAL1* and *CDKN2A/2B* [61−63]. A strong association of *FTO* gene with type 2 diabetes was replicated in different studies, but this was weakened after an adjustment of BMI, indicating that the association with T2D was mediated by BMI [64−66]. On the other hand, recent studies have pointed out that a variant in *FTO* was associated with risk of type 2 diabetes independently of its observed effect on BMI [67,68]. Large-scale type 2 diabetes case−control studies in which cases and controls are matched at the individual level for BMI, as well as body fat content or body adiposity index, may help to investigate whether the *FTO* variant would cause type 2 diabetes [69]. Also, stratification of type 2 diabetes cases by BMI would be helpful

TABLE 29.2 Type 2 Diabetes Susceptibility Loci With Genome-Wide Significance ($p < 5 \times 10^{-8}$)

Nearby Gene	Variant	Chromosome	Risk Allele	Other
FAF1	rs17106184	1	G	A
MACF1	rs2296172	1	G	A
NOTCH2	rs10923931	1	T	G
PROX1	rs340874	1	C	T
ATP8B2	rs67156297	1	A	G
COBLL1	rs7607980	2	T	C
THADA	rs7578597	2	T	C
GCKR	rs780094	2	C	T
BCL11A	rs243021	2	A	G
RBMS1	rs7593730	2	C	T
TMEM163	rs6723108	2	T	G
RBM43, RND3	rs7560163	2	C	G
GRB14	rs3923113	2	C	A
IRS1	rs2943641	2	C	T
DNER	rs1861612	2	T	C
CCDC85A	rs1116357	2	G	A
ASB3	rs9309245	2	G	C
ADAMTS9	rs4607103	3	C	T
ADCY5	rs11708067	3	A	G
IGF2BP2	rs4402960	3	T	G
PPARG	rs1801282	3	C	G
UBE2E2	rs6780569	3	G	A
PSMD6	rs831571	3	C	T
ST64GAL1	rs16861329	3	G	A
LPP	rs6808574	3	C	T
TMEM154	rs6813195	4	C	T
WFS1	rs10010131	4	G	A
MAEA	rs6815464	4	C	G
ARL15	rs702634	5	A	G
ZBED3	rs4457053	5	G	A
ANKRD55	rs459193	5	G	A
PAM, PPIP5K2	rs35658696	5	G	A
SSR1, RREB1	rs9505118	6	A	G
POU5F1-TCF19	rs3130501	6	G	A
CDKAL1	rs7754840	6	C	G
ZFAND3	rs9470794	6	C	T
KCNK16	rs1535500	6	T	G

(*Continued*)

TABLE 29.2 (Continued)

Nearby Gene	Variant	Chromosome	Risk Allele	Other
JAZF1	rs864745	7	T	C
GCC1, PAX4	rs6467136	7	G	A
GCK	rs4607517	7	A	G
DGKB, TMEM195	rs2191349	7	T	G
KLF14	rs972283	7	G	A
MIR129, LEP	rs791595	7	A	G
TP53INP1	rs896854	8	T	C
SLC30A8	rs13266634	8	C	T
ANK1	rs515071	8	C	T
TLE1	rs2796441	9	G	A
TLE4 (CHCHD9)	rs13292136	9	C	T
CDKN2A/B	rs10811661	9	T	C
GLIS3	rs7041847	9	A	G
GPSM1	rs11787792	9	A	G
PTPRD	rs17584499	9	T	C
DMRTA1	rs1575972	9	T	A
CDC123, CAMK1D	rs12779790	10	G	A
HHEX, IDE	rs1111875	10	C	T
TCF7L2	rs7903146	10	T	C
ZMIZ1	rs12571751	10	A	G
GRK5	rs10886471	10	C	T
VPS26A	rs1802295	10	A	G
DUSP8	rs2334499	11	T	C
MTNR1B	rs10830963	11	G	C
CENTD2 (ARAP1)	rs1552224	11	A	C
KCNJ11	rs5219	11	T	C
KCNQ1	rs2237892	11	C	T
INS-IGF2	rs11564732	11	G	A
MIR4686	rs7107784	11	G	A
CCND2	rs11063069	12	G	A
MPHOSPH9	rs4275659	12	C	T
TSPAN8, LGR5	rs7961581	12	C	T
HMGA2	rs1531343	12	C	G
HNF1A	rs7957197	12	T	A
KLHDC5	rs10842994	12	C	T
CCDC63	rs11065756	12	G	A
FAM60A	rs147538848	12	A	G
SGCG	rs9552911	13	G	A
SPRY2	rs1359790	13	G	A

(*Continued*)

TABLE 29.2 (Continued)

Nearby Gene	Variant	Chromosome	Risk Allele	Other
ZFAND6	rs11634397	15	G	A
PRC1	rs8042680	15	A	C
C2CD4A/B	rs7172432	15	A	G
RASGRP1	rs7403531	15	T	C
HMG20A	rs7178572	15	G	A
AP3S2	rs2028299	15	C	A
INAFM2	rs67839313	15	C	T
FTO	rs8050136	16	A	C
BCAR1	rs7202877	16	T	G
HNF1B (TCF2)	rs4430796	17	G	A
SRR	rs391300	17	G	A
SLC16A13	rs312457	17	G	A
SLC16A11	rs13342692	17	C	T
MC4R	rs12970134	18	A	G
BCL2	rs12454712	18	T	C
LAMA1	rs8090011	18	G	C
GATAD2A/CILP2/PBX4	rs3794991	19	T	C
PEPD	rs3786897	19	A	G
GIPR	rs8108269	19	G	T
HNF4A	rs4812829	20	A	G
DUSP9	rs5945326	X	A	G

in identifying additional risk variants, especially in lean cases that may have a stronger genetic predisposition to type 2 diabetes [70].

The first wave of type 2 diabetes GWAS was followed by a second wave combining existing or new GWAS into larger number of participants. In 2008, the DIAGRAM (Diabetes and Genetics Replication and Meta-Analysis) consortium performed meta-analysis of three previously published GWAS to augment power to detect additional loci of similar or smaller effect additional loci [71]. This study identified new loci including *JAZF1*, *CDC123-CAMK1D*, *TSPAN8-LGR5*, *THADA*, *ADAMTS9*, and *NOTCH2* [71]. Also, the first two GWAS of type 2 diabetes among East Asians were conducted in 2008, which identified Asian specific association with *KCNQ1* (potassium voltage-gated channel, KQT-like subfamily, member 1) [72,73]. Association of *KCNQ1* variant with type 2 diabetes was not previously detected in studies of European populations due to much lower risk allele frequency in Europeans than East Asians (5% vs 40%). The subsequent round of meta-analysis by the DIAGRAM consortium identified an independent signal in *KCNQ1* among Europeans [74].

In 2009, two additional diabetes loci, *IRS1* (insulin receptor substrate 1), which is related to insulin resistance and hyperinsulinemia [75], and *MTNR1B* (melatonin receptor 1B), which is related to impaired early insulin secretion [76,77] were identified. Qi et al. identified *RBMS1* (RNA binding motif single-stranded interacting protein 1) gene in a nested case–control study of two prospective cohorts in a discovery stage, and 11 studies in a replication stage [78]. The DIAGRAM consortium further identified 12 additional diabetes susceptibility loci such as *HNF1A* (HNF1 homeobox A) and *HMGA2* (high-mobility group AT-hook 2) by combining GWAS data from 8130 individuals with type 2 diabetes and 38,987 controls of European descent and following up previously unidentified meta-analysis signals in a further 34,412

cases and 59,925 controls [74]. Subsequently, MAGIC (the Meta-Analyses of Glucose and Insulin-related traits Consortium) investigators combined data from multiple GWAS of traits of glucose and insulin metabolism, and found five new loci, *ADCY5* (adenylate cyclase 5), *PROX1* (prospero homeobox 1), *GCK*, *GCKR* (glucokinase (hexokinase 4) regulator), and *DGKB* (diacylglycerol kinase β)-*TMEM195* (transmembrane protein 195), as well as replicated known signals at *TCF7L2* and *SLC30A8* [79]. Since the identified single-nucleotide polymorphisms (SNPs) for type 2 diabetes are considered to be markers for the functional variant responsible for the observed effect, additional fine-mapping of the loci in even larger sample sets is necessary. To do this cost-efficiently, Metabochip, a custom genotyping array, was developed to examine variants nominally associated with type 2 diabetes and other metabolic traits and to perform fine-mapping of established loci [80]. This cost-effective Metabochip contributed to genotyping many additional samples and increasing the power of meta-analysis. A meta-analysis of Metabochip data by the DIAGRAM consortium identified 10 novel type 2 diabetes susceptibility loci in the European population [81]. In addition, large-scale meta-analyses in multiethnic population-based studies from European, African-American, Hispanic-Latino, and Asian studies using a gene-based CardioChip array identified type 2 diabetes-associated variants in *BCL2* [82]. Further GWASs across different ethnicities have identified additional type 2 diabetes loci [83–89]; some population-specific genetic variants, such as variant in the *SLC16A11* among Hispanic/Latino population, were also identified [90].

To better understand the genetic basis of type 2 diabetes susceptibility, genome-wide trans-ancestry meta-analysis was conducted in a large number of participants among 26,488 cases and 83,964 controls of multiple ethnicities (European, East Asian, south Asian, and Mexican and Mexican American). The trans-ethnic meta-analysis increases the power to detect new candidate loci and enhances the fine-mapping resolution of causal variants because of differences in the structure of linkage disequilibrium (LD) between diverse populations through combining GWAS across different ancestry groups. By following up the strongest signals of association from the trans-ethnic meta-analysis among additional 21,491 cases and 55,647 controls of European ancestry, seven new susceptibility loci of type 2 diabetes were recently identified [91].

VI GWAS OF TYPE 2 DIABETES-RELATED QUANTITATIVE TRAITS

GWASs of type 2 diabetes-related quantitative traits have also discovered more than 70 loci associated with glucose homeostasis [79,92–100] (Fig. 29.2). The MAGIC investigators performed a large meta-analysis of 21 discovery GWAS cohorts followed by targeted replication of 25 loci in 33 additional cohorts [79]. They identified associations of SNPs in or near *ADCY5* (adenylate cyclase 5), *MADD* (MAP kinase activating death domain), *ADRA2A* (adrenoceptor alpha 2A), *CRY2* (cryptochrome 2 [photolyase-like]), *FADS1* (fatty acid desaturase 1), *GLIS3* (GLIS family zinc finger 3),

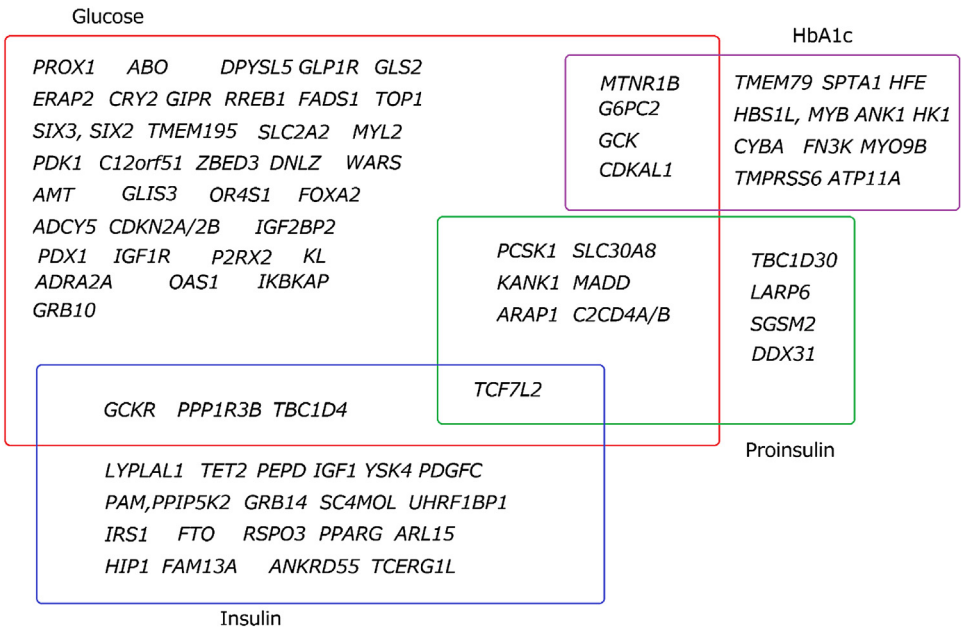

FIGURE 29.2 Loci associated at genome-wide significance with glucose homeostasis.

SLC2A2 (solute carrier family 2 member 2), *PROX1* (prospero homeobox 1), and *C2CD4B* (C2 calcium-dependent domain containing 4B) with fasting glucose and one locus near *IGF1* (insulin-like growth factor 1) associated with fasting insulin and HOMA-IR [79]. They also replicated associations of variants in *GCK*, *GCKR*, *G6PC2* (glucose-6-phosphatase catalytic subunit 2), and *MTNR1B* with fasting glucose. Most of the identified type 2 diabetes loci are related to insulin secretion and β-cell function, while fewer loci are implicated in insulin resistance [79]. For example, *GCKR* is reported to be associated with fasting insulin and insulin resistance, as well as fasting glucose and type 2 diabetes [101,102]. In more recent studies, including larger sample sizes and also considering effect of BMI, more genetic variants associated with insulin sensitivity were identified [99,100]. Some loci associated with glucose homeostasis also showed associations with type 2 diabetes, though the loci affecting these two outcomes do not completely overlap. In a study of 53 glycemic loci, 33 were also associated with an increased risk of type 2 diabetes [99]. Fig. 29.3 shows per-allele β-coefficients for glucose and insulin concentrations versus ORs for type 2 diabetes resulting from meta-analysis investigating associations

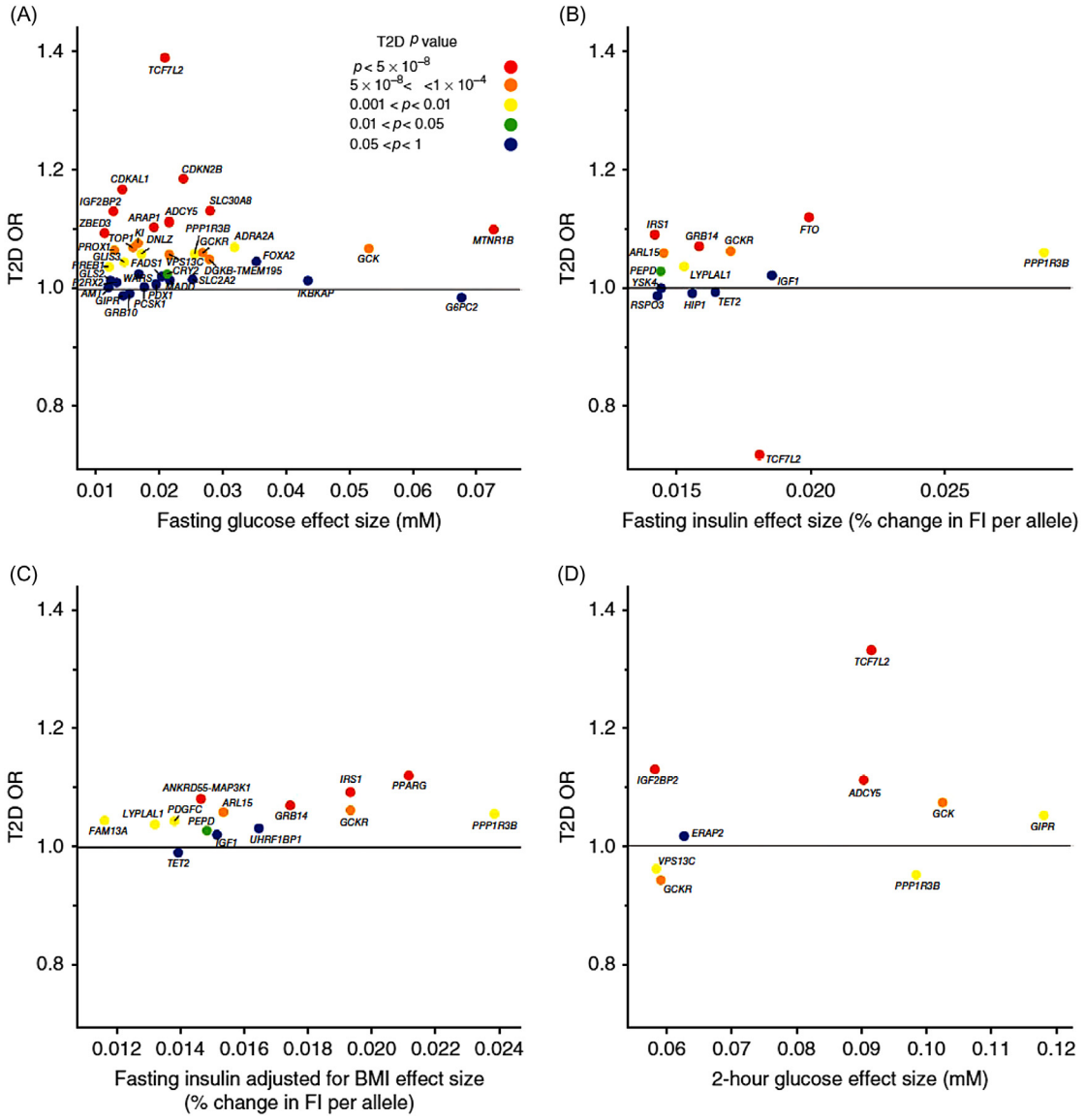

FIGURE 29.3 Per-allele β-coefficients for glucose and insulin concentrations versus ORs for type 2 diabetes (T2D). Panel (A): fasting glucose concentration versus T2D; panel (B): fasting insulin (FI) concentration versus T2D; panel (C): fasting insulin concentration adjusted for BMI versus T2D; and panel (D): 2-hour glucose versus T2D. *From R.A. Scott, V. Lagou, R.P. Welch, et al., Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat. Genet. 44 (2012) 991–1005.*

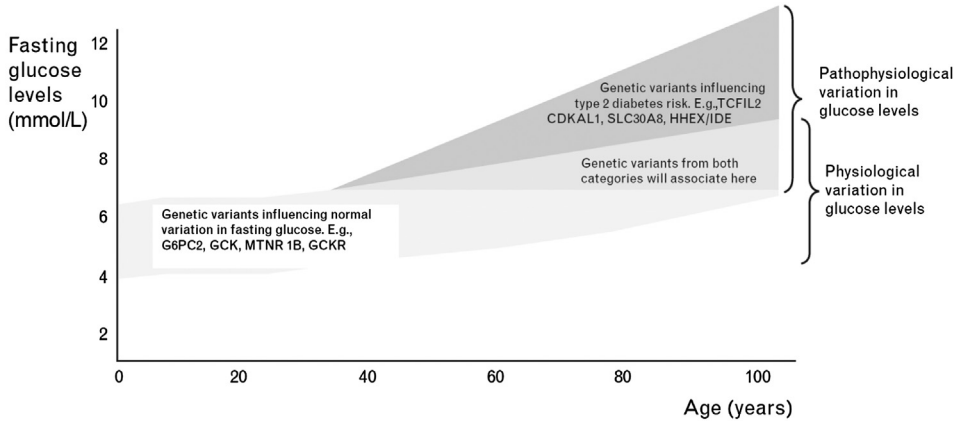

FIGURE 29.4 A schematic representation of how genetic variants influence fasting glucose concentrations in the normoglycemic population and genetic variants that influence the risk of type 2 diabetes may be observed in different glycemic states. Genes determining the normal variation in fasting glucose levels within the normoglycemic population differ from the genes that influence fasting glucose to rise above the normal level leading to type 2 diabetes. There is a gray zone in between these two extremes where people with glucose concentrations approaching the diabetes range (>7 mmol/L) may be either at the top end of the normal range of physiological glucose that shifts slightly upward with age, or at the bottom end of the pathophysiological range and on their way to diabetes. *From N.M. De Silva, T.M. Frayling, Novel biological insights emerging from genetic studies of type 2 diabetes and related metabolic traits, Curr. Opin. Lipidol. 21 (2010) 44–50.*

with glycemic traits [99]. The glucose-raising allele was associated with increased risk of type 2 diabetes, although effect sizes for fasting glucose and type 2 diabetes were weakly correlated (Fig. 29.3A) [99]. Also, a total of 13 among 19 loci associated with fasting insulin concentration showed associations with type 2 diabetes, with the insulin-raising allele associated with higher risk of type 2 diabetes, except at *TCF7L2* (Fig. 29.3B and C), where the allele associated with lower fasting insulin was associated with higher fasting glucose levels [99]. In this study, some SNPs associated with 2-hour glucose were also associated with type 2 diabetes, although the 2-hour glucose-raising alleles at *PPP1R3B*, *GCKR*, and *VPS13C-C2CD4A-C2CD4B* showed associations of decreasing risk of type 2 diabetes (Fig. 29.3D) [99]. The results indicate that not all effects of genetic variants on concentrations of glucose variations in healthy individuals would translate to the risk of type 2 diabetes (Fig. 29.4).

Among studies included in the MAGIC meta-analysis, investigators performed a cluster analysis of effects of 36 type 2 diabetes loci [103] and showed that there were five clusters of loci that appear to have primary effects on: (1) insulin sensitivity (*PPARG*, *KLF14*, *IRS1*, *GCKR*); (2) reduced insulin secretion and fasting hyperglycemia (*MTNR1B*, *GCK*); (3) insulin processing (*ARAP1*); (4) insulin processing and secretion without a detectable change in fasting glucose levels (*TCF7L2*, *SLC30A8*, *HHEX/IDE*, *CDKAL1*, *CDKN2A/2B*); and (5) a final group which contained 20 risk loci with no clear-cut associations to continuous glycemic traits. More studies are warranted to understand the functional alterations underlying genetic associations.

VII FINE-MAPPING OF IDENTIFIED TYPE 2 DIABETES LOCI

Type 2 diabetes loci are mainly identified in European ancestry, partly taking advantage of long-range LD among Europeans. However, the discovered loci usually contain more than one variant affecting diabetes risk, and one could be causal. To distinguish a causal variant from many nearby variants associated with type 2 diabetes, trans-ethnic fine-mapping in multiple ethnic groups has been performed by leveraging the differences in the structure of LD between different populations [91,104]. A large-scale trans-ethnic study was conducted by the DIAGRAM Consortium investigators and they showed improvement in the resolution of fine-mapping of common variant association signals through trans-ethnic meta-analysis [91]. In a recent study of East Asian, European, South Asian, African-American, and Mexican American descent, comprehensive trans-ancestral fine-mapping of four established type 2 diabetes susceptibility loci (*CDKAL1*, *CDKN2A-B*, *IGF2BP2*, and *KCNQ1*) was conducted [104]. These studies provide further evidence to understand the biology of specific susceptibility loci [91,104].

The DIAGRAM Consortium investigators also performed fine-mapping of 39 established type 2 diabetes loci in 27,206 cases and 57,574 controls of European ancestry, and their data suggested potential mechanisms underlying the association between variations in *MTNR1B* gene and type 2 diabetes risk [105]. Melatonin plays a key role in adjusting the biological clock, and MTNR1B has been associated with fasting glucose and risk of type 2 diabetes [76,77,106]. An epidemiological study also

showed significant association between lower melatonin secretion and a higher risk of developing type 2 diabetes [107]. In human islets and liver, the risk allele of rs10830963 at the *MTNR1B* gene creates a binding site for NEUROD1, increasing FOXA2 enhancer activity; this increased MTNR1B expression might contribute to development of type 2 diabetes [105,108].

VIII SEQUENCING AND RARE VARIANTS

Many of the GWAS-identified common variants confer only modest to small effects on risk of type 2 diabetes, and it has been considered that low-frequency ($0.005 < \text{MAF} \leq 0.05$) and rare ($\text{MAF} \leq 0.005$) variants may explain the majority of heritability of type 2 diabetes [109] since these variants are not tagged by conventional genome-wide genotyping arrays [44]. Rare variant association studies have become possible methods because the price of sequencing has dramatically fallen, along with advances of next-generation sequencing technologies. Nonetheless, deep whole-genome sequencing (WGS) remains expensive to perform for large numbers of individuals, and alternative approaches to discover these variants include targeted sequencing, exome sequencing, low-depth WGS (when combined with imputation), and extreme-phenotype sampling. For example, array-based genotyping and exome sequencing in Greenlandic population revealed a nonsense p.Arg684Ter variant (allele frequency of 17%) in *TBC1D4* associated with higher concentrations of 2-hour glucose and insulin [110].

In a recent study of a large numbers of individuals with and without type 2 diabetes, whole-genome and exome sequencing, together with genotyping and imputation, was performed to increase statistical power, and the authors tested whether lower-frequency variants explained much of the reaming heritability of type 2 diabetes [111]. In their results, variants associated with type 2 diabetes after sequencing were overwhelmingly common and most fell within regions previously identified by GWAS [111]. These data suggest that combination of genome and exome sequencing in large number of participants may provide limited evidence to document the role of lower-frequency variants in predisposition to common form of type 2 diabetes [111].

IX GENETIC RISK SCORE AND PREDICTION MODEL FOR TYPE 2 DIABETES

Use of genetic risk score (GRS) and genetic prediction models, which are calculated on basis of GWAS-identified multiple SNPs, has become a common approach to represent genetic predisposition in many studies [112]. A GRS combining multiple SNPs may be useful for screening individuals with a genetically high risk for type 2 diabetes [113,114]. Also, it has been found that a GRS for type 2 diabetes might be predictive of cardiovascular complications among patients with type 2 diabetes [115].

In a Danish population-based Inter99 study, a GRS combining 46 variants was predictive of 5-year changes in plasma glucose and β-cell function, as well as the development of type 2 diabetes [116]. The effect of the GRS on 5-year changes in fasting plasma glucose was stronger among individuals who increased their BMI in the study [116]. A longitudinal study of Chinese adults with a 9-year follow-up also suggested that a GRS combing 40 SNPs was predictive of glucose deterioration through its effect on deteriorating β-cell function [117].

Nonetheless, clinical utility of the GRS of type 2 diabetes has been an issue, especially when compared with classical risk factors (such as clinical measurements and demographic variables including family history of diabetes). The discriminatory ability of a GRS for diabetes, which is usually assessed by area under the curve of ROC, is limited [118,119]. On the other hand, the GRS in combination with classical risk factors, such as obesity and family history of diabetes, showed competence in identifying subgroups with a particularly high risk for type 2 diabetes in the Health Professionals Follow-up Study (HPFS) and Nurses' Health Study [113]. Individuals with a positive family history of diabetes and a GRS in the highest quintile had a nine times higher risk of type 2 diabetes compared with those without a family history of diabetes and with a GRS in the lowest quintile (Fig. 29.5) [113]. In another study, GRS derived using 65 SNPs into a classical risk model (Framingham Offspring Study type 2 diabetes risk model) slightly improved discrimination and reclassification for type 2 diabetes [119]. Clinical utility of genetic variants can be further investigated when more diabetes-related genetic variants are identified.

X GENE–ENVIRONMENT INTERACTIONS IN TYPE 2 DIABETES AND DIABETES-RELATED TRAITS

Most of the variants identified so far explain only a small proportion of heritability of type 2 diabetes, which is so-called "missing" heritability [109]. The missing heritability of type 2 diabetes could be partly explained by interactions between the genetic variations and lifestyle factors [120–122]. Increasing studies have shown gene–environment interactions on type 2 diabetes and diabetes-related traits. In the HPFS, there were significant interactions between the diabetes GRS and the Western

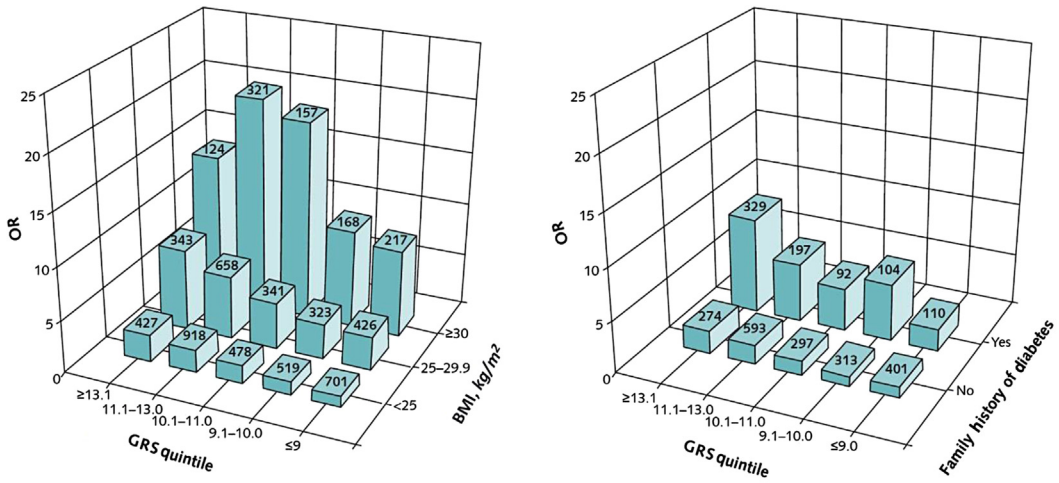

FIGURE 29.5 Joint effects of conventional risk factors and GRS on risk for type 2 diabetes. Values on bars indicate sample size. Left: Joint effects of body mass index and GRS (adjusted for age and sex) from pooled data for men and women. Right: Joint effects of family history of diabetes and GRS (adjusted for age, sex, and body mass index) from pooled data for men and women. *From M.C. Cornelis, L. Qi, C. Zhang, et al., Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry, Ann. Intern. Med. 150 (2009) 541–550.*

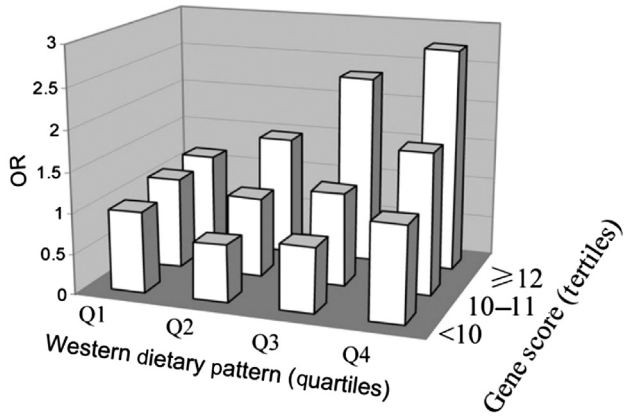

FIGURE 29.6 ORs of diabetes risk according to joint association of Western dietary pattern scores (in quartiles; Q) and GRSs. The ORs were adjusted for age, BMI, smoking, alcohol consumption, physical activity, family history of diabetes, and total energy intakes. *From L. Qi, M.C. Cornelis, C, Zhang, et al., Genetic predisposition, Western dietary pattern, and the risk of type 2 diabetes in men, Am. J. Clin. Nutr. 89 (2009) 1453–1458.*

dietary pattern, which were characterized by high intakes of red meat, processed meat, and refined foods [123]. The Western dietary pattern showed a significant association for increased risk of diabetes among men with a higher GRS, but not among those with a low GRS [123] (Fig. 29.6). In the Malmö Diet and Cancer Study, dietary fiber intake modified an association of *TCF7L2* genotype (rs7903146) and incidence of type 2 diabetes; higher fiber intake was associated with a low risk of type 2 diabetes among persons without the risk allele (p interaction = 0.049) [124]. Results of the National Health and Nutrition Examination Surveys suggest that carbohydrate intake may modify the associations of *CDKAL1* rs4712523 and *FTO* rs8050136 with type 2 diabetes among non-Hispanic whites [125]. A meta-analysis of 14 cohort studies of Europeans from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium showed significant interactions of *GCKR* rs780094 genotype and whole-grain intake on fasting insulin concentrations ($p = 0.006$), where higher whole-grain intake was associated with less reduction in fasting insulin concentrations among individuals with insulin-raising allele [126]. Zinc is an essential micronutrient found in most foods and it is important for β-cell function and glucose homeostasis. A meta-analysis of cohort studies showed that higher total zinc intake may attenuate the glucose-raising effect of *SLC30A8* variant rs11558471 [127]. In results of the Preventing Overweight Using Novel Dietary Strategies (POUNDS Lost) trial, which is a 2-year randomized diet intervention trial among overweight and obese individuals, *PCSK7* genotype modified an association of dietary carbohydrate intake on changes in insulin sensitivity [128].

The Diabetes Prevention Program (DPP) is a multicenter randomized clinical trial to investigate whether lifestyle intervention or metformin therapy would prevent or delay the onset of diabetes among individuals at high risk for the disease [129]. Results of the DPP suggest that the lifestyle intervention was effective in individuals with higher genetic risk of diabetes [130]. Individuals with diabetes-risk-increasing TT genotype of *TCF7L2* variant rs7903146 were 1.55 times more likely to develop diabetes than those without the T allele [131]. Such association

was stronger in the placebo group than in the lifestyle intervention group, suggesting that lifestyle modification might attenuate the genetic effect, although test of interaction was not statistically significant [131]. On the other hand, for improvement of insulin sensitivity, metformin treatment and lifestyle modification improve insulin sensitivity independent of insulin-resistance GRS [132]. While an increasing number of studies has investigated the gene−environment interaction in type 2 diabetes and its related traits, characterizing details of interactions using currently available statistical approaches is still challenging because of requisite for a sufficient sample size with prospective design to assess the incidence of type 2 diabetes, a multiple testing issue to test a large number of hypotheses, and measurement errors, especially for the assessment of dietary intake. Also, a few studies are accompanied by adequate replication data or compelling mechanistic explanations [133]. Some patients with diabetes may change their dietary habits before the clinical diagnosis of diabetes, and thus well-designed large prospective cohort studies collecting genetic and detailed lifestyle factors would be warranted to study the gene−environment interactions in type 2 diabetes.

XI CHALLENGE AND FUTURE DIRECTION

Much progress has been made to reveal genetic architecture for type 2 diabetes, and nearly 100 loci for type 2 diabetes have been identified. Nonetheless, their roles in genetic prediction, actual causal variants, and biological functions remain to be explored. With continued efforts on fine-mapping studies, it is likely that a full picture of variants of type 2 diabetes can be evaluated and introduced for disease prediction in clinical settings. Future efforts to characterize the role of gene−environment interactions as well as other epigenetic modifications are also needed. Epigenetic and structural variation studies, as well as integrating data on various omics [134], would help identify new variants that may be important in type 2 diabetes risk prediction.

XII SUMMARY

Both genetic and nongenetic factors contribute to the risk of developing type 2 diabetes—genetic background determines susceptibility to insulin resistance and β-cell dysfunction, and nongenetic factors such as overweight or obesity, unhealthy dietary habits, and physical inactivity exacerbate the inherited abnormalities [135]. Traditional genetic research, GWAS, and sequencing studies have provided consistent evidence on the genetic basis of type 2 diabetes and glycemic traits, and have identified a group of variants through application of meta-analysis across diverse populations and genotype imputation approaches. Advances in sequencing have led to the discovery of potentially functional variants. Future continuing efforts will contribute to identifying additional loci, investigate the pathogenic roles of the variants and genes involved, and explore their interactions with environmental factors in determining the risk of type 2 diabetes.

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Chapter 30

Obesity and the Risk for Type 2 Diabetes

George A. Bray

Pennington Biomedical Research Center/LSU, Baton Rouge, LA, United States

I INTRODUCTION

A Obesity

Obesity is a worldwide problem affecting more than 100 million Americans [1,2]. The prevalence of overweight and obesity has been on the rise since 1980 and now affects 68.5% of the adult population [3,4]. The presence of obesity or overweight is associated with a number of diseases, of which diabetes mellitus is one of the most important, but it also predicts future hypertension, heart disease, obstructive sleep apnea, and many other comorbidities. Preventing the twin time bombs of obesity and diabetes has major importance for the future of health care worldwide [5]. Whether the associated disorders are present or not, the patient with obesity should be encouraged to lose weight by appropriate methods because this will reduce the risk of developing future diabetes, and weight loss, if sufficient, may reverse diabetes that already exists [6].

The first steps in management of body weight are self-directed approaches (diet, physical activity, support groups, and commercial programs with an evidence base to support efficacy). Many patients succeed with these strategies alone. For patients who struggle, and who have medical issues that will improve with more weight loss, there are hospital and community-based lifestyle intervention programs and medically supervised weight management programs that employ adjunctive medications, surgical devices, and surgical procedures.

B Diabetes

The prevalence of diabetes mellitus, like the prevalence of obesity, continues to rise worldwide since obesity is in large measure driving the increase in diabetes. In the United States, approximately 86 million people have prediabetes, and one in three people have the metabolic syndrome [7]. Individuals with prediabetes are at high risk for conversion to type 2 diabetes and 3−11% will convert to type 2 diabetes annually [8]. As the rise in prevalence of obesity has slowed over the past 5 years, there has also been a gradual but sustained decrease in the rate of diabetes occurring from 2008 to 2014 [9].

II DIAGNOSTIC CRITERIA FOR OBESITY AND DIABETES

A Obesity

People who are overweight or obese are most commonly classified using the body mass index or BMI which is the weight in kilograms divided by the square of the height in meters. It is a valuable tool for estimating overweight and obesity in populations, since it correlates with total fat mass [10], but it has significant limitations when applied to individual patients [11]. A classification based on BMI is shown in Table 30.1. When evaluating a patient with obesity, the health care provider should also measure the waist circumference, particularly in patients with a BMI between 25 and 35 kg/m^2 [13]. In addition, evaluation of components of the metabolic syndrome should be considered [12]. A BMI of greater than 25 kg/m^2 is defined as overweight, and a BMI of 30 kg/m^2 or more is defined as obesity (Table 30.1). Padwal et al. [14] evaluated nearly 50,000 women and 5000 men who had body fat estimated by dual energy x-ray absorptiometry (DXA) and BMI and found that both a low BMI and a high percentage of body fat increased mortality in both men and women.

B Central Adiposity and Visceral Fat

An increase in visceral fat increases risk for diabetes, heart disease, and cancer. Either the waist circumference or waist circumference divided by hip circumference (WHR) can be

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TABLE 30.1 A Classification of Obesity Based on BMI

Category	Class	BMI Range
Normal range		18.5–24.9 kg/m^2
Overweight		25–29.9 kg/m^2
Obese		>30 kg/m^2
	1	30–34.9 kg/m^2
	2	35–39.9 kg/m^2
	3 (Extreme)	>40 kg/m^2

Within these BMI categories, additional personal risk assessment is needed because degree of risk can vary.
Source: Adapted from M.D. Jensen, D.H. Ryan, C.M. Apovian, J.D. Ard, A.G. Comuzzie, K.A. Donato, et al., 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society, J. Am. Coll. Cardiol. 63 (25 Pt B) (2014) 2985–3023 [12].

TABLE 30.2 Criteria for the Metabolic Syndrome

Criterion	Men	Women
Waist circumference using national criteria—U.S. criteria	>40 in. (>102 cm)	>35 in. (>88 cm)
Triglycerides	>150 mg/dL	>150 mg/dL
HDL-Cholesterol	<40 mg/dL	<50 mg/dL
Glucose	100–125 mg/dL	100–125 mg/dL
Blood pressure	>135/85	>135/85

Source: Adapted from K.G. Alberti, R.H. Eckel, S.M. Grundy, P.Z. Zimmet, J.I. Cleeman, K.A. Donato, et al., Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity, Circulation 120 (16) (2009) 1640–1645 [15] and Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III), JAMA 285 (2001) 2486–2497 [16].

used as surrogates to assess the amount of central adiposity. A waist circumference greater than 102 cm (40 in.) in men or greater than 88 cm (35 in.) in women is the current American threshold for increased metabolic risk, but for other populations a waist circumference >80 cm for women and >94 cm for men may be more appropriate. Measurement of waist circumference is used in the definition of the "metabolic syndrome," which is a predictor of risk for developing diabetes and heart disease. The criteria for the Metabolic Syndrome are shown in Table 30.2. An individual with this syndrome is at higher risk of developing diabetes and cardiovascular disease (CVD). However, central adiposity can also be measured more precisely using magnetic resonance imaging or computed tomography. When this is done, visceral adipose tissue is shown to be a stronger predictor of diabetes and CVD than subcutaneous adipose tissue [17]. In a study of 15,184 adults aged 18–90 years from NHANES III database, persons with normal weight and central obesity, defined by high WHR, had the worst long-term survival. For example, a man with a normal BMI (22 kg/m^2) and central obesity had an 87% greater risk of mortality than an individual with a similar BMI but no central obesity, and this man had twice the mortality risk of participants who were overweight or obese according to BMI only. Women with normal-weight central obesity also had a 48% higher mortality risk than women with a similar BMI but no central obesity and those who were obese according to BMI only. Expected survival estimates were consistently lower for those with central obesity when age and BMI were controlled for [18].

C Diabetes Mellitus

Diabetes mellitus is a metabolic disorder with elevated concentrations of blood glucose secondary to insulin resistance and defective insulin secretion from the beta-cells of the pancreas [19,20]. Asymptomatic diabetes, particularly type 2 diabetes mellitus, may be diagnosed by abnormally high blood glucose levels during routine health examinations (Table 30.3). Chronic hyperglycemia and abnormal lipid levels are associated with pathologic and functional changes in several organ systems including the eyes, the blood vessels, the heart, and the kidneys [21]. Diabetes can be subdivided into several types, including type 1 or juvenile-onset diabetes, type 2 often called adult-onset diabetes, and gestational diabetes mellitus. Type 1 diabetes is an autoimmune disease with destruction of the β cells of the pancreas, which leads to a deficiency of insulin. Type 2 diabetes is most often seen in adults and is due to a combination of insulin resistance and a deficiency in insulin secretion (Fig. 30.1). Deficiencies in insulin secretion often manifest themselves during pregnancy and lead to a diagnosis of "gestational diabetes" which may resolve. However, these women are at high risk for future type 2 diabetes mellitus.

III EPIDEMIOLOGICAL EVIDENCE FOR A RELATIONSHIP OF OBESITY AND DIABETES

There is overwhelming evidence from epidemiological studies in cohorts of individuals from many countries using both cross-sectional and longitudinal methods that

TABLE 30.3 Diagnostic Reference Values for Diabetes

Parameters	Group Criteria	Normal[a]	Prediabetes	T2DM
Hemoglobin A1c	ADA WHO	<5.7%‡ <6.0%§	5.7–6.4%‡ 6.0–6.4%§	≥6.5%
Fasting plasma glucose	ADA WHO	<100 mg/dL‡ <110 mg/dL§	100–125 mg/dL‡ 110–125 mg/dL§	≥126 mg/dL
Two-hour plasma glucose from an oral GTT		<140 mg/dL	140–199 mg/dL	≥200 mg/dL

[a]*Normal glucose metabolism;* ‡*American Diabetes Association;* §*World Health Organization.*
Oral GTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus; ADA, American Diabetes Association; WHO, World Health Organization.
Source: Adapted from R.A. DeFronzo, E. Ferrannini, L. Groop, R.R. Henry, W.H. Herman, J.J. Holst, et al., Type 2 diabetes mellitus, Nat. Rev. 1 (2015) 1–22 [8].

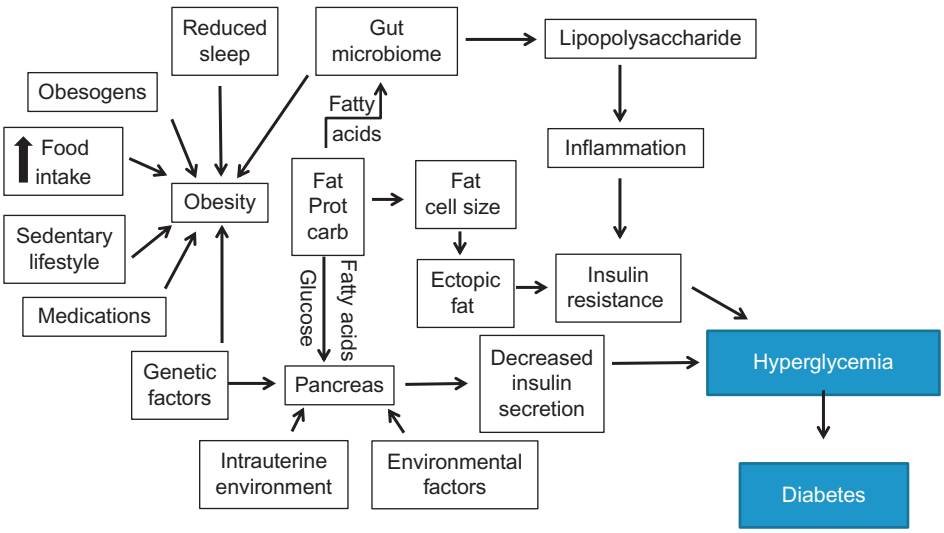

FIGURE 30.1 A model of relation of obesity to development of diabetes.

BMI or central adiposity, and the increase in body weight over time predict future diabetes. One of the most provocative depictions of the relationship of obesity and diabetes is the maps from the Center for Disease Control and Prevention, which show the rising prevalence of obesity preceding rising prevalence of diabetes from 1985 to 2015 [22].

A meta-analysis of prospective studies provided evidence that as upper body adiposity increases, the presence of the metabolic syndrome and the risk of developing diabetes both increase [23]. The duration of obesity in younger compared with older individuals is associated with a greater risk for diabetes [24]. Weight gain in adult life increases the risk of developing diabetes, particularly in the age range 25–40 years [25].

The duration of an increased body weight also increases the risk of diabetes. In the National Longitudinal Survey, a total of 8157 adolescents and young adults aged 14–21 years in 1979 with self-reported measures of height, weight, and diabetes status (type unspecified) were followed from 1981 through 2006. For a given level of excess BMI-years, younger individuals compared with older ones and Hispanic and black compared with white individuals had higher risk of developing diabetes [26].

Foods can increase or decrease the risk of diabetes [27,28]. Coffee, either caffeinated or decaffeinated, reduces the risk of developing type 2 diabetes [29,30] and this risk is associated with an inverse relationship between sex-hormone-binding globulin and the risk of diabetes [31], and it may be that these two are related. The risk of diabetes is also reduced among those with higher consumption of nuts and seeds [32], whole grains [33,34] and in those who consume yogurt [35]. The risk for diabetes is increased by consumption of either processed or unprocessed red meat [36] by consumption of more than 1 egg per day [37] and consumption of sugar-sweetened beverages [38]. Some of these relations of foods with risk of

diabetes may be reflected in the increase in body weight, with sugar-sweetened beverages being the most obvious example [39]. Among these foods consumption of red meats is associated with a 4-year weight gain. In contrast yogurt is associated with weight loss over the 4-year follow-up [40].

A How Does Obesity Increase the Risk of Developing Diabetes?

Not everyone who becomes obese develops diabetes, suggesting that there must be additional genetic and/or environmental factors that are involved. To organize this brief discussion, Fig. 30.1 depicts a "model" of the factors that are involved in producing obesity and how these and other factors lead some individuals with obesity to develop diabetes.

Many individuals with obesity have no "associated" findings such as prediabetes, dyslipidemia, hypertension, or other findings and have been referred to as "Healthy Obese" [41]. However, it is now clear from long-term follow-up studies that half or more of these individuals will develop obesity-related disease during their lifetime [42]. Thus, prevention and treatment remain important.

We can identify a number of factors that produce obesity. The major ones are food intake that is higher than needed for the level of energy expenditure that persists over months to years [11]. An additional important dietary factor is the intake of sugar-sweetened beverages [43]. Some genes have major effects on the risk of obesity [44,45], but a much larger number of genes, possibly more than 100, have minor effects [46,47]. Reduced sleep time is associated with higher risk of obesity [48]. A number of drugs can cause weight gain [49]. The microbiome may play a role, but its relative importance is still unsettled [50,51]. The human microbiota consists of up to 100 trillion microbes that exist in a largely symbiotic relationship with their human hosts, and carry at least 150 times more genes (the microbiome) than are present in the entire human genome. Effects of the microbiota on adiposity are noted following inoculation of germ-free mice with the microbiota from normal mice. In human beings, inoculation of mice with the microbiota from monozygotic (MZ) and dizygotic (DZ) human twin pairs discordant for obesity shows a progressively greater increase in both fat mass and fat-free mass in animals receiving microbes from the obese twins, despite no significant differences between groups in daily chow consumption [52].

The vaginally delivered neonate is initially colonized with the vaginal and distal gut bacteria of the mother, while babies delivered by cesarean section (C-section) are initially colonized predominantly with the skin bacteria from the mother [53,54]. Thus, vaginally delivered neonates have relatively and absolutely larger populations of Bacteroides and Bifidobacteria species than those born by C-section [55]. These differences may persist for months to years [56]. The observations that increased Bacteroidetes populations are present in both obesity and in children born by C-section (seven from Rosenbaum) suggest that the infant microbiome may contribute to the 40% increased risk of obesity in children [57] and young adults [58,59] delivered by C-section.

In most [60,61], but not all [62], studies, both the diversity of the microbiota and the fractional proportion of Bacteroidetes species relative to Firmicutes are decreased in obese versus lean individuals. These proportions are extremely sensitive to caloric balance in subjects studied before, during, and after weight loss while ingesting the same liquid formula diet of identical macronutrient composition [63].

In humans, Kocelak et al. [64] examined resting energy expenditure (REE), body composition, and the gut microbial population in 50 obese and 30 lean healthy weight-stable subjects. They reported that the obese subjects had a significantly greater total microbial count without significant differences in the ratio of Bacteroidetes/Firmicutes, both of which had been reported to be lower in obese individuals in other studies [60,61]. Over the entire group of subjects, the size of the population of Firmicutes was positively correlated with fat mass and negatively correlated with REE and the maximal oxygen consumption (VO_{2max}). Total bacterial count was significantly positively correlated with VO_2 and negatively correlated with VCO_2. In multiple regression analysis including fat mass, none of these correlations between the microbiota and energy expenditure remained significant [64].

Obesity is one of the strongest predictors of the risk for diabetes. Type 2 diabetes, like obesity, has a strong heritable component. It has long been recognized that family history plays an important role in determining if one will develop diabetes. Most prospective studies of individuals with prediabetes demonstrate that they are hyperinsulinemic [65] or have impaired insulin secretion. The diabetes-related gene list, however, is different from the list of genes that are related to obesity. All genes can only account for about 85% of the variance in diabetes [8]. If the mother has diabetes, the risk is higher than if the father has it, and the risk rises substantially if two other siblings have diabetes. The single nucleotide polymorphism for TCF7L2 has the strongest association with type 2 diabetes [66,67]. Genome-wide association studies have identified more than 100 common variants, mostly on "introns," and the function is understood for only a few of these "genes." Those with some mechanism include GCKR which encodes the

glucokinase regulatory protein [68,69], KCNJ11 which encodes an ATP-dependent potassium channel, and SLC30A8 which encodes a zinc transporter that is required to store insulin [70].

B Intrauterine Environment—the Barker Hypothesis and Fetal Origins of Type 2 Diabetes and Obesity

Among the environmental/physiological mechanisms that predispose some people with obesity to develop diabetes are events occurring during intrauterine and early postnatal life. Individuals who were malnourished while in utero may be more prone to insulin resistance and diabetes and weight gain than those who are well nourished. This hypothesis often called the "Developmental Origin of Disease" is also called the "Barker Hypothesis" from the name of the man who first described it [71]. Maternal undernutrition forces the fetus to adapt during its intrauterine development and drives a reprogramming of its endocrine−metabolic state to produce permanent changes in the structure and the physiology of key organ systems [71,72]. These changes in low-birth-weight infants (normal range, 3000−4000 g) contribute factors to chronic diseases such as type 2 diabetes, coronary heart disease, stroke, and hypertension in adult life [73].

Infants born to mothers with diabetes tend to have higher birth weight (macrosomia) but are also at higher risk for developing the metabolic syndrome and type 2 diabetes as adults [74−77]. The inverse association between birth weights and diabetes remains significant even after adjustment for adult adiposity. The increased relative risk was seen among lean, overweight, and obese women, indicating that in utero growth has independent effects from adult body weight on the risk for developing type 2 diabetes. However, the greatest risk remains for women of low birth weight who develop obesity as adults [72].

C Maternal Obesity and Gestational Weight Gain

Maternal obesity alters metabolic adjustments and fetal growth during the neonatal period [78,79]. Women with obesity tend to have larger placentas and bigger babies, which are significant predictors of type 2 diabetes in the offspring. One-third of pregnant women in the United States are obese, with increased metabolic complications, preeclampsia, fetal anomalies, and poor pregnancy outcomes. Risk of gestational diabetes is increased by up to eightfold in severe obesity and increases in women who had gained weight within 5 years prior to pregnancy [80]. Both large and small for gestational age infants are seen more often in the obese compared with normal-weight mothers [81]. Several factors are implicated in maternal obesity including: (1) maternal hypertension and its effect on placental size; (2) a subclinical inflammatory state with elevated cytokine [82] and leptin levels; and (3) an enhanced insulin resistance that exaggerates the pregnancy-associated increase in circulating plasma glucose, lipids, and amino acids, exposing the fetus to an excess of all fuel sources.

Guidelines for maternal weight gain were provided in the 1990 Institute of Medicine's (IOM) gestational weight gain which was revised in 2009 (Table 30.4). Reduced gains of 5−9 kg were recommended for mothers who have obesity [83]. But, less than one-third of pregnant women meet those recommendations [84] and excessive weight gains of >19 kg have become common and account for a growing proportion of cesarean deliveries [85] and postpartum retention of body weight [86]. The risk of having an overweight child at age 3 years among 1044 mother−child pairs increased fourfold in mothers whose gestational weight gain was adequate or excessive compared with mothers whose weight gain was inadequate using the 1990 IOM guidelines. The increased risk was independent of parental BMI, maternal glucose tolerance, breast-feeding duration, fetal and infant growth, and

TABLE 30.4 Institute of Medicine Recommendations for Weight Gain During Pregnancy

Prepregnancy BMI	Total Weight Gain		Rates of Weight Gain	
	Range in Kg	Range in Lbs	Mean Range in Kg per Week	Mean Range in Lbs per Week
Underweight (<18.5 kg/m^2)	12.5−18.2	28−40	0.51 (0.44−0.58)	1 (1−1.3)
Normal weight (18.5−24.9 kg/m^2)	11.5−16	25−35	0.42 (0.35−0.50)	1 (0.8−1)
Overweight (25.0−29.9 kg/m^2)	7−11.5	15−25	0.28 (0.23−0.33)	0.6 (0.5−0.7)
Obese (≥30.0 kg/m^2)	5−9	11−20	0.22 (0.17−0.27)	0.5 (0.4−0.6)

Source: Adapted from National Academy of Medicine (2009).**Weight** Gain During **Pregnancy**: Reexamining the Guidelines. Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM **Pregnancy Weight** Guidelines; Rasmussen KM, Yaktine AL, editors. Washington (DC): National Academies Press (US); **2009**.

child behaviors including frequency of watching TV or consuming fast-foods. Efforts at weight control should avoid strict energy and carbohydrate restrictions to avoid ketosis and associated metabolic complications, which can have negative effects on fetal cognitive development [78]. Treatment of maternal obesity aims to improve insulin sensitivity in the mother without reducing fetal glucose levels and compromising fetal growth.

D Smoking During Pregnancy

Women who smoke during pregnancy increase the risk of their offspring developing obesity out to 25 years. This effect is present even if smoking is terminated during the first trimester. The simplest explanation is that nicotine from smoking enters the brain and alters the function of neurons related to future development of obesity [11].

Diabetes develops when the secretion of insulin from the pancreas is no longer sufficient to overcome the barriers to the action of insulin which are lumped together in the term "insulin resistance" [8,87]. Development of type 2 diabetes thus depends on changes in one or both of these two principal variables—insulin sensitivity and insulin secretion. As insulin resistance develops, that is, responsiveness to insulin decreases, an individual's risk of diabetes increases unless they can also increase insulin secretion. Thus, obesity must modify one of these two variables.

E Beta-Cell Function

Insulin resistance is one of the first defects to be identified in individuals who will develop diabetes. The progression to diabetes is associated with failure of the beta-cells in the pancreas to keep up with the demand. Multiple factors contribute to the failure of the beta-cells including resistance to incretin hormones (GIP and GLP-1), lipotoxicity, glucotoxicity, aging, genetic abnormalities, increased secretion of islet amyloid polypeptide, reactive oxygen stress, and inflammation [8]. About 60% of the islet mass consists of beta-cells that secrete insulin in response to glucose, arginine, free fatty acids, and neural stimulation. In diabetes, the mass of beta-cells is reduced, probably by apoptosis. In addition, chronic exposure to high levels of glucose may blunt the responsiveness of these cells to incretins [88] and thus their ability to keep up with the demand for insulin to facilitate glucose uptake peripherally. The improvement of beta-cell function following bariatric surgery implies that these cells have been inhibited and can be resuscitated by reducing the flow of nutrients through the individual that need to be metabolized.

F Insulin Resistance and Inflammation

Insulin resistance can be identified earlier than failure of insulin secretion and is not always associated with the development of diabetes when islet cell secretion can keep up with the demand. Insulin resistance is characterized by reduced clearance of glucose as well as reduced response at the cellular membrane (Fig. 30.1). A prospective study that followed second- and third-generation Japanese Americans for up to 10 years confirmed that the amount of intra-abdominal fat plays an important role in the development of diabetes [89]. In this study, visceral adiposity, measured by computed tomography, was predictive of diabetes incidence, regardless of age, sex, family history of diabetes, fasting insulin, insulin secretion, glycemia, and total and regional adiposity. By increasing the demand for insulin, insulin resistance becomes a risk factor for diabetes, causing glucose intolerance in subjects who have impaired insulin secretory capacity and a reduction in the glucose potentiation of insulin secretion. Incremental insulin response to an oral glucose challenge, an assessment of insulin secretion, suggests that a failure in β-cell function preceded the onset of diabetes. Insulin resistance was also associated with a blunted suppression of glucagon secretion by glucose in impaired glucose tolerance, suggesting that β-cell dysfunction and locally reduced insulin concentrations may exaggerate glucagon secretion because the α-cell becomes less sensitive to glucose.

Insulin resistance occurs not only in muscle and liver, but also in most other tissues including the fat cell, kidney, brain, pancreas, and GI track. The defect in muscle has been extensively studied, but the details are beyond the scope of this chapter. In the muscle, there are defects in insulin signaling at the membrane, in glucose transport, in glucose phosphorylation, and in glycogen synthesis, as well as in the pyruvate dehydrogenase complex and in the oxidative machinery in the mitochondrion [8]. Gluconeogenesis in the liver is a major factor in the fasting hyperglycemia of diabetes, but there is also increased renal gluconeogenesis. Gluconeogenesis is the result of multiple endocrine disturbances including insulin resistance and deficiency, increased glucagon levels, and enhanced delivery of substrates (fatty acids, lactate, glycerol, and amino acids) to the liver.

Another factor that may explain why insulin resistance worsens in some obese individuals and not in others as their fat mass increases is the overproduction by specific adipocytes of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). In a meta-analysis, including 10 prospective studies, with a total of 19,709 participants and 4480 cases, there was a significant dose−response association between IL-6 levels and type 2 diabetes risk. For c-reactive protein (CRP), the

meta-analysis included 22 cohorts, with a total of 40,735 participants and 5753 cases. Elevated CRP levels, like IL-6 levels, were significantly associated with increased risk of type 2 diabetes [90]. This inflammatory process may be set in motion in part by the ingestion of a high-fat diet [91]. In obese individuals, macrophages, referred to as classically activated (M1) macrophages, accumulate in adipose tissue. This may be precipitated by the effect of the high-fat diet on entry of lipopolysaccharide from the intestine into the circulation. These macrophages release cytokines such as IL-1β, IL-6, and TNF-α creating a proinflammatory environment that blocks adipocyte insulin action, contributing to the development of IR and type 2 diabetes mellitus. The Nod-like receptor family, pyrin domain containing 3 (Nlrp3) inflammasome, may play a particularly important role in risk for developing diabetes, since knocking down this gene reduces the inflammatory response to a high-fat diet.

There is evidence (reviewed in DeFronzo et al. [8]) that postabsorptive skeletal muscle fat oxidation in insulin-resistant states is decreased [92]. This decrease is explained by the presence in muscle of carbohydrate-derived malonyl-CoA, which inhibits carnitine palmitoyl transferase, blocking the entry of free fatty acids into the mitochondria. Carnitine palmitoyl transferase activity has been shown to be reduced in the vastus lateralis muscle of insulin-resistant obese individuals. The excess free fatty acids may increase long-chain acyl-CoA concentrations and diacylglycerol, leading to the accumulation of lipids in muscle—an effect conducive to alterations of insulin signaling and insulin action.

IV PREVENTION OF DIABETES BY PREVENTING OBESITY

As noted by DeFronzo et al. [8], a healthy diet, maintaining a BMI $<25\ kg/m^2$, and regular exercise for at least 30 minutes per day could prevent up to 90% of the cases of type 2 diabetes. In the discussion to follow we will focus on prevention and treatment of obesity is a major strategy in this "war" on the prevention of diabetes. One obvious corollary of the fact that obesity is a major risk factor for the development of diabetes is that encouraging individuals at risk for diabetes to lose weight might delay or prevent the development of diabetes. Thus, weight loss might mitigate the effects of diabetes [93].

Five well-designed clinical trials summarized in Table 30.5 have evaluated the effect of lifestyle modification on the risk for developing diabetes. They include (1) the Da Qing Study [94], (2) the Finnish Diabetes Prevention Study [95–97], (3) the Diabetes Prevention Program (DPP, 2002) [110], (4) the Indian Diabetes Prevention Program [99], and (5) and a Japanese Diabetes Prevention study [100].

A Lifestyle Programs

In the Finnish Diabetes Prevention study, subjects were randomized to a control group which was given general information on diet and exercise, or to an intervention group which received detailed counseling by dietitians to help them reduce weight, decrease the intake of fat and saturated fat, and to increase fiber and physical activity. After 3.2 years of follow-up, the lifestyle intervention was associated with a significant reduction in weight (−4.2 vs −0.8 kg) and waist circumference (−4.4 vs −1.3 cm) compared to the control group [95]. There was also a significant reduction in 2-hour plasma glucose and serum insulin, serum triglycerides, and blood pressure. The risk of developing diabetes was reduced by 58% in the intervention group compared to the control group. At 7-year follow-up, participants in the intervention group still had a 43% lower diabetes risk (Lindstrom et al., 2013). In spite of these positive findings, 10-year total mortality and cardiovascular mortality rates did not differ between the control and intervention groups [111]. The number of deaths, however, was only 16 (3%) and the rate was low compared with 11% in another Finnish cohort that had received no intervention.

The DPP was conducted in 27 centers around the United States. Participants were randomly assigned to one of four groups: (1) an intensive lifestyle group with a 7% weight loss goal and a goal of 150 minutes/week of physical activity, (2) metformin (850 mg/day for 1 month, increasing to 850 mg bid if tolerated), (3) a group receiving troglitazone which was terminated prematurely due to side effects from the drug, and (4) a placebo group receiving a pill that "looked" like the metformin pill [98,112]. After 2.8 yearrs of follow-up, average weight loss was 5.6, 2.1, and 0.1 kg in the lifestyle, metformin, and placebo groups, respectively. This produced a 58% reduction in the incidence of type 2 diabetes in the lifestyle group. Interestingly, this is the same figure observed in the Finnish Diabetes Prevention study (Tuomilheto et al., 2001). The lifestyle intervention also resulted in improvements in hypertension, triglycerides, and HDL cholesterol, and the differential between these three main treatment groups persisted for 10 years [110].

B Medication

Medication can also reduce the incidence of diabetes among individuals with impaired glucose tolerance. There are eight randomized controlled trials that have examined the effects of pharmacological agents on the prevention of type 2 diabetes. In the DPP, metformin at a dose of

TABLE 30.5 Interventions to Delay or Prevent the Conversion from Prediabetes to Diabetes

Study	Total Randomized	Population	Duration (Year)	Intervention	Risk Reduction
Lifestyle Interventions					
Chinese Da Qing Study	577	IGT	6	Group diet/exercise	38%
Finnish Diabetes Prevention Study	522	IGT, BMI ≥25 kg/m^2	3.2	Individual diet/exercise	58%
Diabetes Prevention Program	2161	IGT, BMI ≥24, FPG >95 mg/dL (5.3 mmol/L)	2.8	Individual diet/exercise	58%
Indian Diabetes Prevention Program	531	IGT	2.5	Individual diet/exercise	29%
Japanese trial	458	IGT (men), BMI = 24	4	Individual diet/exercise	67%
Pharmacotherapy					
DPP (Diabetes Prevention Program) Metformin Arm	2155	IGT, BMI ≥24, FPG >95 mg/dL (5.3 mmol/L)	2.8	Metformin 850 mg/day	31%
STOP NIDDM: Study to Prevent Noninsulin-Dependent Diabetes	1429	IGT, FPG >100 mg/dL (5.6 mmol/L)	3.2	Acarbose (300 mg)	25%
Voglibose Trial (α-glucosidase inhibitor)	1780	IGT	3	Voglibose 0.2 mg thrice a day	41%
XENDOS: Xenical in the Prevention of Diabetes in Obese Subjects	3305	BMI >30	4	Orlistat (360 mg)	37%
TRIPOD: Troglitazone in Prevention of Diabetes	266	Previous GDM	2.5	Troglitazone (400 mg)	55%
DREAM: Rosiglitazone	5269	IGT or IFG	3	Rosiglitqazone (8 mg)	60%
DPP (Diabetes Prevention Program) Troglitazone Arm	1167	IGT or IFG	0.9	Troglitazone	75%
ACT NOW ACTOS (Pioglitazone) in the Prevention of Diabetes	502	IGT or IFG	2.4	Pioglitazone (45 mg)	72%
SEQUEL (Topiramate/ Phentermine ER or Qsymia)	475	IGT and/or metabolic syndrome	2	Topiramate/ phentermine ER 7.5/46 and 15/92 mg	70% 79%
Bariatric Surgical Intervention					
SOS (Swedish Obese Subjects)	3239*	Obese volunteers for bariatric surgery	Up to 15	Surgery compared to usual care	82%

Nonrandomized group: Bariatric patients ($N = 1658$) volunteered and were matched as closely as possible to a control group ($N = 1771$). *SOS was a "matched" control group not a "randomized" study. The total number included the operated and matched control group. Papers in this table—*Lifestyle*: Pan et al. [94]; Tuomilehto et al. [95]; Knowler et al. [98]; Ramachandra et al. [99]; Kosaka et al. [100]; *Medication*: Metformin: Knowler et al. [98]; DPP [101]; Chiasson et al. [102]; Kawamori et al. [103]; Torgerson et al. [104]; Buchanan et al. [105]; Gerstein et al. [106]; Knowler [107]; DeFronzo et al. [108]; Garvey et al. [13]; *Bariatric Surgery*: Sjostrom et al. [109].

850 mg/day reduced the incidence of diabetes by 31% [98]. Metformin also produced weight loss which was related to the degree of adherence to the drug regimen [101]. Orlistat, an inhibitor of intestinal lipase, reduced the conversion rate from impaired glucose tolerance to diabetes by 37% [104]. Acarbose, a blocker of starch digestion, reduced the incidence by 25% [102]. Another starch blocker voglibose was also shown to have the same effect [103]. Three different thiazolidinediones have been shown to produce a very significant reduction in the risk

of developing diabetes. In the DPP, troglitazone reduced the incidence of new cases of diabetes by 55% [107]. Pioglitazone in the ACT NOW trial reduced the incidence of diabetes by 72% [108]. Rosiglitazone in the DREAM trial reduced the incidence of diabetes by 60% [106]. Most recent data from the combined use of topiramate and phentermine have been shown to have a significant reduction in risk of conversion to diabetes that is related to the degree of weight loss [113]. Table 30.5 summarizes therapies proven to be effective in pharmacotherapy trials.

The final strategy is the use of bariatric surgery that can prevent the development of diabetes in individuals with prediabetes and reverse diabetes that is already present. It was Pories et al. [114] who suggested that the weight loss with bariatric surgery might reverse diabetes and reduce the risk of its development. This was most convincingly established in the Swedish Obese Subjects (SOS) study. The SOS study evaluated the conversion from prediabetes to diabetes [109]. They found that the conversion was reduced by 82%, one of the largest that has been reported. Surgery can also produce a remission from diabetes. Among randomized controlled trials, the percentage of remission from diabetes to nondiabetes after surgery was 92% with a range of 85–97% [115].

In summary, this chapter has examined the relationship between obesity and the risk of developing diabetes. The epidemiological data are very strong and there are clear-cut mechanisms involving insulin resistance and beta-cell failure that can account for the changes. The proof of the pudding in this relationship is that weight loss by any one of several means, or reduction in insulin resistance (thiazolidinediones), can significantly reduce the risk of converting to diabetes.

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FURTHER READING

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Chapter 31

The Role of Diet in the Prevention and Treatment of Diabetes

Judith Wylie-Rosett[1] **and Linda M. Delahanty**[2]

[1]Albert Einstein College of Medicine, Bronx, NY, United States, [2]Massachusetts General Hospital Diabetes Center, Boston, MA, United States

I INTRODUCTION

The number of people in the United States with diabetes is estimated to be 29.1 million or 9.3% of the population based on the 2010 U.S. Census population estimates and diabetes prevalence data from the 2009−12 National Health and Nutrition Examination Survey, the 2009−12 National Health Interview Survey, the 2012 Indian Health Service data, and 2012 U.S. population estimates [1,2]. However, only 21.1 million of those with diabetes have diagnosed diabetes, and the other 8.1 million are unaware they have diabetes. The risk of having diabetes increases with age, and 25.9% of the U.S. population aged 65 years or older have diabetes. In addition, 37% (86 million) of U.S. adults (≥20 years of age) are at high risk for developing diabetes and can be classified as having prediabetes [1,2].

Data from 219 countries and territories were used to estimate the global prevalence of diabetes for 2013 and projections for 2035 [3]. The number of adults (age 20−79 years) with diabetes was estimated to be 382 million (8.3% of the world population) in 2013, with an increase to 592 million (8.8% of the world population) by 2035. The increase between 2013 and 2035 is expected to be 55% globally. The largest proportional increase is expected in low-income countries (108%); middle-income countries are expected to be intermediate (51%), and the high-income countries are expected to have the smallest increase (28%). Countries with a large population and rapid economic development are likely to carry the greatest diabetes burden. For example, China and India rank first and second, respectively, for number of diabetes cases, with the U.S. ranking third. The projected number of adults with diabetes in 2035 is 143 million in China, 109 million in India, and 30 million in the United States [3].

The increased global diabetes burden appears to be the result of population aging and urbanization, with the associated changes in diet and physical activity. However, there is growing evidence that in India and China, the prevalence in rural areas is approaching that in urban areas, perhaps related to mechanization [4]. The estimated direct medical cost for prevention and treatment of diabetes worldwide was approximately $376 billion in 2010 and will exceed $490 billion by 2030 [5]. Total costs are substantially higher due to loss of productive years of life, particularly in developing countries. The effort to reduce the growing public health burden of diabetes in the United States and throughout the world includes goals for prevention as well as treatment.

This chapter provides an overview of criteria for diagnosing and classifying diabetes. Prevention of diabetes is addressed focusing on weight management and increased physical activity. The review of treatment focuses on medical nutrition therapy (MNT). Diabetes MNT for preventing complications focuses on the goal of achieving as close to normal metabolism as possible. Metabolic goals target controlling blood pressure and lipid levels as well as glycemic control. The role of MNT in treating diabetes complications is also addressed. The chapter concludes with an overview of programs and resources for preventing and treating diabetes.

II DIAGNOSTIC CRITERIA AND DIABETES CATEGORIES

Diagnostic tests and criteria used to identify individuals who are at risk for developing diabetes (prediabetes) or who have diabetes [6] are listed in Table 31.1. The

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TABLE 31.1 Assessment Methods and Criteria for Diabetes Diagnoses

Assessment Method	At Increased Risk for Diabetes (Prediabetes)	Diagnostic for Diabetes	GDM (At Least Two of the Following)
Hemoglobin A1c[a]	A1c 5.7–6.4%	≥ 6.5%	Not applicable
Fasting plasma glucose (FPG)	Impaired fasting glucose of 100–125 mg/dL (5.6–6.9 mmol/L)	FPG ≥ 126 mg/dL (7.0 mmol/L) (on two occasions)	FPG ≥ 95 mg/dL (5.3 mmol/L)
Oral glucose tolerance test, 75 g glucose load	Impaired glucose tolerance 2-hour glucose of 140–199 mg/dL (7.8–11.0 mmol/L)	2-Hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L)	1-Hour plasma glucose ≥ 180 mg/dL (10.0 mmol/L) 2-Hour plasma glucose ≥ 155 mg/dL (8.6 mmol/L) 3-Hour plasma glucose ≥ 140 mg/dL (7.8 mmol/L)
Symptomatic	Not applicable	Symptoms of hyperglycemia or hyperglycemic crisis with random plasma glucose ≥ 200 mg/dL (11.1 mmol/L)	Not applicable

[a]*National Glycohemoglobin Standardization Program certified and standardized to the DCCT assay.*

classifications of diabetes include type 1, type 2, gestational, and diabetes due to other causes [6].

Type 1 diabetes, formerly known as insulin-dependent or juvenile diabetes, represents approximately 5–10% of known cases of diabetes [6]. Type 1 diabetes is characterized by severe insulin deficiency requiring exogenous insulin to prevent ketoacidosis, coma, and death. Population-based research in the United States from the SEARCH for Diabetes in Youth Study multicenter observational study of youth has assessed the incidence of clinically diagnosed diabetes [7]. Data from 2002 and 2003 suggest that approximately 15,000 youth are newly diagnosed with type 1 diabetes annually in the United States [7]. The incidence of type 1 diabetes is higher in whites than in other ethnic/racial groups, with the greatest discrepancy at younger ages [7].

Type 2 diabetes, formerly known as noninsulin-dependent or adult-onset diabetes, accounts for the vast majority (90–95%) of diabetes cases [8]. Development of type 2 diabetes is associated with insulin resistance and inadequate pancreatic cell compensatory insulin production [6]. Symptoms and signs associated with developing type 2 diabetes, which are usually more subtle than for type 1 diabetes, may be related to the presence of complications and include poor wound healing, blurred vision, recurrent gum or bladder infections, and changes in hand or foot sensation. Type 2 diabetes may remain undiagnosed for several years before a clinical diagnosis is made. Many individuals are asymptomatic, and their glucose elevation may be detected as the result of a routine blood test. Type 2 diabetes is rare in children younger than the age of 10 years, but it accounts for an increasing proportion of cases as age increases [7].

Gestational diabetes (GDM) is defined as hyperglycemia identified during pregnancy [8], but for many women undiagnosed type 2 diabetes may be identified during pregnancy. The diagnosis and management of GDM is addressed in a separate chapter.

Specific types of diabetes due to other causes include monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation) [6]. Any disorder or compound that affects the function of the cell in the pancreas can cause diabetes. Endocrinopathies increase counterregulatory hormone production (e.g., acromegaly [excess growth hormone] and Cushing's syndrome [excess cortisol] can cause diabetes if there is insufficient compensatory insulin production). Steroids and second-generation antipsychotic medications, which increase insulin resistance and visceral fat, may increase insulin requirements beyond endogenous capacity. Cystic fibrosis-related diabetes is the most common comorbidity in people with cystic fibrosis. The addition of diabetes to cystic fibrosis is associated with poorer nutrition outcomes in addition to more severe inflammatory lung disease. However, the increase in mortality-associated diabetes in cystic fibrosis patients has decreased with advances in treatment [6].

III MNT FOR DIABETES PREVENTION AND TREATMENT

The American Diabetes Association (ADA) has stated that "medical nutrition therapy (MNT) is an integral component of diabetes prevention, management,

and self-management education" [8] and that "individuals who have diabetes should receive individualized MNT as needed to achieve treatment goals, preferably provided by registered dietitian (RD) familiar with the components of diabetes MNT" [9]. These recommendations are based on evidence that individualized MNT is effective in decreasing hemoglobin A1c (A1c) levels in patients diagnosed with prediabetes [10]. Research data from type 1 and type 2 diabetes have demonstrated a decrease in A1c of −0.3 to −1% in patients with type 1 diabetes and −0.5 to −2% in patients with type 2 diabetes [8,9]. The evidence review for the recommendations noted that randomized controlled trials have demonstrated the effectiveness of nutrition therapy in improving various markers of cardiovascular and hypertension risk. MNT provided by an RD for patients with abnormal lipid profiles has been shown to "reduce daily fat (5−8%), saturated fat (2−4%) and energy intake (232−710 kcal/day) and lower triglycerides (11−31%), LDL cholesterol (7−22%) and total cholesterol (7−21%) levels" [9]. Medicare and other third-party payers require that MNT be administered by a licensed/certified RD or nutrition professional. The steps and strategies for MNT in the prevention and treatment of diabetes are listed in Table 31.2.

The ADA endorses eating a healthful dietary pattern high in fruits, vegetables, whole grains, and nuts; low sodium intake (<2300 mg/day with further reduction in sodium intake individualized for patients with hypertension); and, for most individuals, a modest amount of weight loss [8]. However, the ADA evidence review also noted that a variety of dietary patterns are "modestly effective in managing diabetes including Mediterranean-style, Dietary Approaches to Stop Hypertension (DASH) style, plant-based (vegan or vegetarian), lower-fat, and lower carbohydrate patterns" [9]. Larger scale trials are needed to refine MNT recommendations regarding dietary patterns.

A Prevention of Diabetes

Prevention is an important strategy for reducing health care costs as well as the morbidity and mortality resulting from diabetes. Although there are no established approaches for prevention of type 1 diabetes, research is needed to provide a better understanding of environmental triggers as well as the potential role of immunosuppressive therapy and other possible approaches.

Strategies for the prevention of type 2 diabetes target intervention goals of weight loss and increased physical activity to reduce insulin resistance [11,12]. The evidence that a lifestyle intervention can prevent or delay type 2 diabetes is from randomized clinical trials conducted in people at high risk of developing the disease. These trials demonstrate the critical role of lifestyle intervention addressing excess body weight and physical inactivity [11−16].

The Da Qing Diabetes Prevention Study was a 6-year lifestyle intervention (diet, exercise, or diet plus exercise) in 577 adults with impaired glucose tolerance in China. The results of this study provided preliminary evidence that diet and exercise interventions can lower the conversion to overt diabetes [13]. The 20-year follow-up results showed that individuals in the combined intervention group had a 43% lower incidence of diabetes during the 20-year period and spent an average of 3.6 fewer years with diabetes compared to control group participants [17]. After 23 years of follow-up, this lifestyle intervention was the first to show a reduction in the long-term cardiovascular consequences of diabetes, reducing all-cause and cardiovascular disease (CVD) mortality, mainly among women. The reduction in mortality associated with the lifestyle intervention was mediated by its effect in delaying the onset of diabetes [18].

The Finnish Diabetes Prevention Study (FDPS) was a randomized, controlled clinical trial in 522 individuals with impaired glucose tolerance randomized to either a control or an intervention group [11]. The lifestyle intervention involved a low-fat (<30% of calories from fat and <10% of calories from saturated fat), high-fiber (at least 15 g/1000 kcal) diet in conjunction with moderate-intensity exercise for at least 30 minutes/day [11]. After an average follow-up of 3.2 years, results of the FDPS showed a 58% reduction in the incidence of type 2 diabetes with lifestyle intervention [19]. There were small but significant reductions in total cholesterol, triglyceride, systolic blood pressure, and measurements of inflammation as well as an increase in high-density lipoprotein (HDL) cholesterol in the lifestyle intervention group but not in the control group [19]. Studies from the FDPS of candidate genes affecting energy metabolism showed the importance of genetic polymorphisms in defining responses to lifestyle interventions [20].

The Diabetes Prevention Program (DPP), which was a randomized, controlled, clinical trial conducted in 3234 ethnically diverse individuals with impaired glucose tolerance, randomized participants to one of three treatments: (1) standard lifestyle recommendations plus metformin, (2) standard lifestyle recommendations plus placebo, or (3) intensive lifestyle intervention [12]. The intensive lifestyle intervention was designed to achieve a 7% weight loss (based on initial body weight) and at least 150 minutes of moderate-intensity physical activity per week. The intervention goals included reducing energy intake (500−1000 fewer calories than the amount needed to maintain baseline weight) with a low-fat (25% of calories from fat) diet. The intensive lifestyle intervention program consisted of a 16-session core curriculum in which participants received individualized advice on how

TABLE 31.2 MNT Steps and Strategies by Class of Diabetes

MNT Steps
1. Nutrition referral (physician): Include medical diagnoses and history, laboratory tests, medications, relative body weight, and other pertinent information. 2. Nutrition assessment (RD): Evaluate referring information, the patient's concerns/questions, and review patient's relevant knowledge, skills, and behavior. 3. Nutrition diagnosis (RD): Develop coded problem list for MNT intervention; for example, "Inconsistent carbohydrate intake and inadequate meal planning with meals and snacks varying from 0 to 150 g of carbohydrates on a regular basis." 4. Nutrition intervention (RD): Teach skills for planning meals and carbohydrate intake, develop goals collaboratively with patient related to inconsistent intake, ask patient about barriers to achieving goals, and develop problem-solving strategies with patient to address barriers. 5. Nutrition monitoring and evaluation (RD): Conduct ongoing review of biochemical factors such as A1c and serum lipid levels, as well as lifestyle factors such as dietary intake.
MNT Strategies
Prediabetes
• If overweight, reduce calorie intake to achieve 5–10% weight loss. • Increase moderate-intensity physical activity to at least 150 min per week. • Reduce and/or modify type of fat to achieve weight and lipid goals.
Type 1 diabetes
• Assess usual lifestyle, focusing on eating and physical activity habits, noting time schedule. • Plan insulin therapy to match insulin action to lifestyle schedule—for example, starting with one unit of insulin for 15 g of carbohydrate. • Monitor blood glucose levels while keeping lifestyle consistent to better assess how to match insulin to carbohydrate intake and physical activity. • Adjust insulin and lifestyle to achieve blood glucose levels in the target range. • Create algorithms for adjusting insulin for lifestyle flexibility (e.g., insulin to carbohydrate ratios) and to correct blood glucose levels that are not in the target range.
Type 2 diabetes
• If overweight, reduce calorie intake to achieve 5–10% weight loss. • Increase physical activity. • Monitor blood glucose to assess pattern of glycemic control. • If postprandial glucose level is high, spread food intake throughout the day (using five or six small meals/snacks rather than having fewer larger ones). • Reduce and/or modify type of fat to achieve weight and lipid goals.
GDM
• Plan calorie intake to achieve desired weight gain based on desirable body weight. • Balance carbohydrate intake throughout the day (usually 40–50% of calories). • Monitor glucose approximately seven times per day; adjust intake to achieve glucose levels in target range. • Add exogenous insulin if target glucose levels are not achieved by diet alone.
Secondary diabetes
• Assess interrelationship between primary disease(s) and its treatment in relation to the secondary diabetes to establish treatment priorities. • Institute diabetes treatment as needed to avoid short- and long-term complications.

to reach their goals with follow-up booster sessions to support maintenance of lifestyle changes [21].

After an average follow-up period of 2.8 years, the DPP results showed that the incidence of diabetes was reduced by 58% in the intensive lifestyle intervention group compared to 31% in the metformin group [12]. Further analysis revealed that weight loss was the dominant predictor of the observed reduced diabetes incidence: when adjusted for changes in diet and physical activity, each kilogram of weight loss resulted in a 16% reduction in diabetes risk [22]. Furthermore, lower percentage of calories from fat and increased physical activity accounted for the weight loss, indicating that it was through these intermediates that the diabetes risk reduction was accomplished [22]. However, participants in the intensive lifestyle intervention who did not achieve

the 7% weight loss goal but did achieve the goal of 150 minutes of physical activity per week had a 44% reduction in the incidence of diabetes [22]. The intensive lifestyle treatment effects did not differ by sex, race, or ethnic group.

Unlike the intensive lifestyle intervention, which was effective across the entire baseline body weight and fasting glucose ranges, metformin was ineffective in those with a body mass index (BMI) less than 30 kg/m^2 and minimally effective in those with a BMI less than 35 kg/m^2 or with a fasting glucose level less than 110 mg/dL (6.1 mmol/L). As in the FDPS [19], insulin sensitivity improved in the intensive lifestyle intervention group, with a smaller increase in the metformin group and no change in the placebo group. Insulin secretion decreased in all groups, but it was associated with improved cell function only in the intensive lifestyle intervention group [23]. The lifestyle intervention resulted in a lower prevalence and need for medical treatment of hypertension and dyslipidemia [24]. The lifestyle intervention also lowered inflammatory biomarkers associated with increased cardiovascular risk [25].

Ten-year follow-up of the DPP found that the cumulative incidence of diabetes remained the lowest in the intensive lifestyle intervention group [16]. After 15 years of follow-up, diabetes incidence was reduced by 27% in the lifestyle intervention group and by 18% in the metformin group, compared with the placebo group. There were no overall differences in microvascular outcomes between treatment groups; however, in women, the lifestyle intervention was associated with a 21% ($p = 0.03$) and 22% ($p = 0.02$) lower prevalence of microvascular complications compared with the placebo and metformin groups, respectively. In addition, participants who did not develop diabetes had a 28% lower prevalence of microvascular complications, again supporting the importance of diabetes prevention [26].

Both the DPP and the FDPS asked participants to self-monitor their food intake [19,21,27]. In the FDPS, participants were asked to complete 3-day food records four times per year [19,27]; in the DPP, participants were asked to self-monitor their activity, food intake, calories, and fat grams daily during the first 24 weeks and then at least 1 week per month thereafter. In the DPP, the frequency of dietary self-monitoring was related to success at achieving both the physical activity goal and the weight loss goal. Moreover, DPP participants, who were 65 years old or older, were more likely to complete self-monitoring records, report a lower percentage of calories from fat, and meet the activity and weight loss goals than were those who were younger than 45 years [28]. Thus, it is not surprising that older participants had a greater (71%) risk reduction in the development of diabetes with lifestyle intervention [12]. Lifestyle coaches in the DPP taught the participants to use a problem-solving approach to manage high-risk situations (stress, vacations, and eating out) and used a toolbox approach to deal with barriers to lifestyle change [21,29]. The DPP lifestyle intervention materials have been made available to all health care practitioners at https://dppos.bsc.gwu.edu/web/dppos/lifestyle. Diabetes prevention intervention materials for community programs are available at http://www.cdc.gov/diabetes/prevention/index.html. When translating the DPP lifestyle intervention into practice, it is important to provide clients with both the knowledge and the skills needed to make lifestyle changes. The skills of goal setting, self-monitoring, problem-solving, relapse prevention, and managing high-risk situations were critical in facilitating the lifestyle change process [29,30].

Dietary patterns that are higher in intake of fruits, vegetables, legumes and nuts, whole grains rather than refined grain, and lower intake of red/processed meats and sugar-sweetened beverages are associated with reduced risk of developing diabetes [31]. Such dietary patterns focus on intake of naturally occurring nutrient-rich food groups with increasing emphasis on the quality of dietary fats and carbohydrates consumed. Meta-analyses of prospective studies have indicated that greater adherence to Mediterranean patterns (higher diet score) resulted in a respective 23% and 19% reduction in risk of developing diabetes [32,33]. Both of the meta-analysis papers noted methodological limitations of the observational studies, and both included the PREDIMED-Reus study [34,35] as the only randomized controlled clinical trial. The PREDIMED-Reus compared the effects of a low-fat dietary pattern (control) to a Mediterranean dietary pattern supplemented with virgin olive oil or mixed nuts on the incidence of diabetes over a 4-year period in a community dwelling with older adults who had three cardiovascular risk factors but did have over diabetes. The PREDIMED intervention did not include any intervention goals related to physical activity or weight loss [34,35]. Relative to the low-fat pattern, the combined Mediterranean patterns resulted in a 30% reduction in the incidence of diabetes after adjusting for confounders that may affect the risk of developing diabetes [34,35].

The HEALTHY study was a randomized, controlled, multicenter study of middle-school-aged children in the United States designed to reduce risk factors associated with developing type 2 diabetes [36]. The combined prevalence (intervention and control schools) of overweight and obesity in schools was decreased (primary outcome), but there were no differences in prevalence of overweight and obesity between intervention and control schools. However, the intervention schools had significantly greater decreases in BMI z-scores, percentage of students with a waist circumference at or above the 90th percentile, fasting insulin levels, and obesity prevalence [37].

The efficacy of the intervention in prevention of type 2 diabetes in youth will require additional participant follow-up and further testing.

B Diabetes Treatment

The overall goal of therapy for diabetes is to normalize metabolism (with emphasis on blood glucose and lipids, particularly low-density lipoprotein [LDL] cholesterol and blood pressure) in order to prevent diabetes-related complications [8]. Acute complications include hyperglycemia resulting in ketoacidosis in type 1 and hyperglycemic coma in type 2 diabetes and hypoglycemia in patients treated with insulin or medications that raise blood insulin levels [8]. Longer term complications include microvascular, macrovascular, and neuropathic complications [8]. Results from clinical trials in individuals with both type 1 and type 2 diabetes provide evidence that improving metabolic control (glycemia, blood pressure, and lipids) greatly reduces the development and progression rates for microvascular, macrovascular, and neuropathic complications [21,27,38–41]. The goals are sometimes referred to as the ABCs, with targets of A1c $\leq$ 7%, blood pressure (systolic $<$ 140 mm Hg and diastolic $<$ 90 mm Hg), and LDL cholesterol $<$ 100 mg/dL [42,43]. These clinical goals are typically achieved with a combination of nutrition and lifestyle interventions and medications.

A range of oral medications, injectables, and insulin are available to manage glycemia in addition to nutrition and lifestyle recommendations (Table 31.3) [42,44]. Type 1 diabetes is primarily managed with intensive insulin therapy. Due to the increasingly large number of antihyperglycemic drugs for type 2 diabetes, the growing uncertainty regarding their proper selection and sequence, and the paucity of comparative effectiveness research on long-term treatment outcomes with many of these medications, the ADA and the European Association for the Study of Diabetes (EASD) position statement focuses individualizing both treatment targets and treatment strategies using a patient-centered approach and shared decision making [44,45].

The target for glycemic control is influenced by a variety of fixed and modifiable patient and disease factors including age, disease duration, life expectancy, important comorbidities, established vascular complications, the risks and consequences to the patient from an adverse drug event, patient attitude, and expected treatment efforts and access to resources and support system. Initial therapy with lifestyle intervention and metformin as monotherapy is recommended for patients with type 2 diabetes due to its low cost, proven safety record, weight neutrality, and possible benefits on cardiovascular outcomes. Assessment of motivation to focus on weight loss and increased activity along with results of self-monitoring of blood glucose is important in determining the need for adjustment or advancement of the medication regimen. If glycemic targets are not achieved within 2 or 3 months or are not sustained, the addition of other medications and transition to new regimens including insulin is recommended with consideration given to the additional complexity and costs of multiple combinations of glucose-lowering medications. MNT and diabetes self-management education are integral to the therapeutic program and ensure that patients have access to information on methods to reduce where possible the amount of medications needed and safely monitor and control blood glucose levels [8].

Diabetes standards of care include routine blood glucose monitoring for all patients with type 1 diabetes and meaningful monitoring for patients with type 2 diabetes based on risk of hypoglycemia [42,46]. Continuous blood glucose monitoring can be useful for patients with type 1 or type 2 diabetes who have unappreciated hyperglycemia, a history of severe hypoglycemia, hypoglycemia unawareness, or an inability to achieve A1c targets. Blood glucose monitoring data can be used as an education tool to help both patients and providers assess the quality of glycemic control, evaluate glucose variability, and interpret blood glucose patterns related to insulin, diet, and activity. Patients who use an insulin pump have a rich data set of additional information to supplement glucose monitoring data that include recorded carbohydrate intake, and time and amount of insulin delivered whether for a meal or correction dose. Nonpump users need to track this information separately [46].

1 Type 1 Diabetes

The Diabetes Control and Complications Trial (DCCT) was conducted in individuals with type 1 diabetes to compare the effects of intensive versus standard glycemic control [47]. Intensive treatment reduced the mean A1c from 9% to 7.2%, and greater attention to dietary strategies accounted for almost one-fourth of the glycemic improvement [47,48]. The risk of the development and progression of retinopathy, albuminuria, and neuropathy was reduced by between 50% and 75% over 8 years [47]. Reduction in the risk of complications was linearly related to the reduction in A1c, indicating that risk reduction can be achieved by improving glycemic control, even if a perfect or normal metabolic state is not achieved [49–51]. These accomplishments, as well as efforts to attenuate the two- or threefold increase in severe hypoglycemia and weight gain, were largely due to educational and nutritional strategies [48,52].

Longer term follow-up of the DCCT cohort in the Epidemiology of Diabetes Interventions and Complications study has documented a continued differential in the risk of microvascular (nephropathy and

TABLE 31.3 Properties of Glucose-Lowering Medications for Treating Diabetes

Class	Compound(s)	Cellular Mechanism(s)	Primary Physiological Action(s)	Advantages	Disadvantages	Cost
Biguanides	• Metformin	Activates AMP-kinase (? other)	• ↓ Hepatic glucose production	• Extensive experience • No hypoglycemia • ↓ CVD events (UKPDS)	• Gastrointestinal side effects (diarrhea, abdominal cramping) • Lactic acidosis risk (rare) • Vitamin B12 deficiency • Multiple contraindications: CKD, acidosis, hypoxia, dehydration, etc.	Low
Sulfonylureas	2nd Generation • Glyburide/ glibenclamide • Glipizide • Gliclazide[a] • Glimepiride	Closes KATP channels on β-cell plasma membranes	• ↑ Insulin secretion	• Extensive experience • ↓ Microvascular risk (UKPDS)	• Hypoglycemia • ↑ Weight • ? Blunts myocardial ischemic preconditioning • Low durability	Low
Meglitinides (glinides)	• Repaglinide • Nateglinide	Closes KATP channels on β-cell plasma membranes	• ↑ Insulin secretion	• ↓Postprandial glucose excursions • Dosing flexibility	• Hypoglycemia • ↑ Weight • ? Blunts myocardial ischemic preconditioning • Frequent dosing schedule	Moderate
Thiazolidinones	• Pioglitazone[a] • Rosiglitazone[b]	Activates the nuclear transcription factor PPAR-γ	• ↑ Insulin sensitivity	• No hypoglycemia • Durability • ↑ HDL-C • ↓ Triglycerides (pioglitazone) • ? ↓ CVD events (PROactive, pioglitazone)	• ↑ Weight • Edema/heart failure • Bone fractures • ↑ LDL-C (rosiglitazone) • ? ↑ MI (meta-analyses, rosiglitazone)	Low
α-Glucosidase inhibitors	• Acarbose • Miglitol	Inhibits intestinal α-glucosidase	• Slows intestinal carbohydrate digestion/ absorption	• No hypoglycemia • ↓Postprandial glucose excursions • ? ↓ CVD events (STOP-NIDDM) • Nonsystemic	• Generally modest HbA1c efficacy • Gastrointestinal side effects (flatulence, diarrhea) • Frequent dosing schedule	Moderate
DPP-4 inhibitors	• Sitagliptin • Vildagliptin[a] • Saxagliptin • Linagliptin • Alogliptin	Inhibits DPP-4 activity, increasing postprandial active incretin (GLP-1, GIP) concentrations	• ↑ Insulin secretion (glucose-dependent) • ↓ Glucagon secretion (glucose-dependent)	• No hypoglycemia • Well tolerated	• Angioedema/urticaria and other immune-mediated dermatological effects • ? Acute pancreatitis • ? ↑ Heart failure hospitalizations	High
Bile acid sequestrants	• Colesevelam	Binds bile acids in intestinal tract, increasing hepatic bile acid production	• ? ↓ Hepatic glucose production • ? ↑ Incretin levels	• No hypoglycemia • ↓ LDL-C	• Generally modest HbA1c efficacy • Constipation • ↑ Triglycerides • May ↓ absorption of other medications	High

(*Continued*)

TABLE 31.3 (Continued)

Class	Compound(s)	Cellular Mechanism(s)	Primary Physiological Action(s)	Advantages	Disadvantages	Cost
Dopamine-2 agonists	• Bromocriptine (quick release)[b]	Activates dopaminergic receptors	• Modulates hypothalamic regulation of metabolism • ↑ Insulin sensitivity	• No hypoglycemia • ? ↓ CVD events	• Generally modest HbA1c efficacy • Dizziness/syncope • Nausea • Fatigue • Rhinitis	High
SGLT2 inhibitors	• Canagliflozin • Dapagliflozin[a] • Empagliflozin	Inhibits SGLT2 in the proximal nephron	• Blocks glucose reabsorption by the kidney, increasing glycosuria	• No hypoglycemia • ↓ Weight • ↓ Blood pressure • Effective at all stages of T2DM	• Genitourinary infections • Polyuria • Volume depletion/hypotension/ dizziness • ↑ LDL-C • ↑ Creatinine (transient)	High
GLP-1 receptor agonists	• Exenatide • Exenatide extended release • Liraglutide • Albiglutide • Lixisenatide[a] • Dulaglutide	Activates GLP-1 receptors	• ↑ Insulin secretion (glucose-dependent) • ↓ Glucagon secretion (glucose-dependent) • Slows gastric emptying • ↑ Satiety	• No hypoglycemia • ↓ Weight • ↓ Postprandial glucose excursions • ↓ Some cardiovascular risk factors	• Gastrointestinal side effects (nausea/ vomiting/diarrhea) • ↑ Heart rate • ? Acute pancreatitis • C-cell hyperplasia/medullary thyroid tumors in animals • Injectable	High
Amylin mimetics	• Pramlintide[b]	Activates amylin receptors	• ↓ Glucagon secretion • ↑ Satiety • Slows gastric emptying	• ↓ Postprandial glucose excursions • ↓ Weight	• Generally modest HbA1c efficacy • Gastrointestinal side effects (nausea/ vomiting) • Hypoglycemia unless insulin dose is simultaneously reduced • Injectable • Frequent dosing schedule • Training requirements	High
Insulins	• Rapid-acting analogs – Lispro – Aspart – Glulisine • Short-acting – Human Regular • Intermediate-acting – Human NPH • Basal insulin analogs – Glargine – Detemir – Degludec[a] • Premixed (several types)	Activates insulin receptors	• ↑ Glucose disposal • ↓ Hepatic glucose production • Other	• Nearly universal response • ↓ Microvascular risk (UKPDS) • Theoretically unlimited efficacy	• Hypoglycemia • Weight gain • ? Mitogenic effects • Injectable • Training requirements • Patient reluctance	Variable

[a]*Not licensed in the United States.*
[b]*Not licensed in Europe for type 2 diabetes.*
CVD, cardiovascular disease; GIP, glucose-dependent insulinotropic peptide; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; MI, myocardial infarction; PPAR-γ, peroxisome proliferator–activated receptor γ; PROactive, Prospective Pioglitazone Clinical Trial in Macrovascular Events; STOP-NIDDM, Study to Prevent Non-Insulin-Dependent Diabetes Mellitus; T2DM, type 2 diabetes mellitus; UKPDS, UK Prospective Diabetes Study.
Source: Adapted from S.E. Inzucchi, R.M. Bergenstal, J.B. Buse, M. Diamant, E. Ele Ferrannini, M. Nauck, et al., Management of hyperglycemia in type 2 diabetes: 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes, Diabetes Care 38 (2015) 140–149, with permission from the American Diabetes Association.

retinopathy) and macrovascular (CVD) complications, even though A1c levels in the two groups have been similar for approximately 8 years [53,54]. After 30 years of follow-up, intensive therapy reduced the incidence of CVD by 30% and the incidence of major cardiovascular events (nonfatal heart attacks, stroke, or cardiovascular death) by 32% [55]. The lower HbA1c levels during the 30-year trial statistically explain all of the observed treatment effect.

Several types of insulin are available (Table 31.3), and they are often used in various combinations in order to match insulin action to the patient's lifestyle—for example, time and type and amount of food eaten. A typical approach in terms of insulin use is a "basal-bolus" regimen, either via subcutaneous insulin infusion (or pump therapy) or via multiple daily injections (MDIs), in which insulin is delivered as a bolus before meals in amounts matched to total carbohydrate intake, with basal insulin on board consistently over time. There are other approaches to MDI that utilize different types of insulin. Patients can either vary their insulin dose to match the amount of food consumed, with additional considerations for physical activity, or meals and physical activity can be held constant in order to match a constant dosing of insulin from day to day. Reduction of insulin dosage is the preferred method of preventing hypoglycemia during and/or after exercise, but this requires planning physical activity ahead of time. For unplanned exercise, increased carbohydrate intake may be needed.

In the DCCT, dietary behaviors associated with better glycemic control in the intensively treated group included adherence to an overall meal plan (timing and amount of carbohydrate), appropriate treatment of hypoglycemia (avoiding excess consumption of carbohydrate to treat symptoms), prompt intervention for hyperglycemia (more insulin and/or less food), and consistent consumption of planned evening snacks [48]. The mean level of weight gain in the intensively treated group was reduced by 50% after the intervention staff focused on strategies to control weight gain. Strategies to minimize potential for weight gain included discussing risk for weight gain prior to initiation of intensive therapy, proactively reducing calorie intake goals by 250−300 calories, adjusting insulin doses preferentially for anticipated increases in activity (rather than adding extra snacks), and avoiding excessive food consumption to prevent and treat hypoglycemia [52].

A strategy to avoid overtreating hypoglycemic symptoms involves (1) documenting that the symptoms are truly indicative of hypoglycemia (blood glucose below the normal fasting range of 70−120 mg/dL), (2) treating the hypoglycemia with 15 g of carbohydrate, and (3) waiting for 15 minutes before eating more. This strategy is sometimes referred to as the 15/15 rule for hypoglycemia. Strategies to minimize the frequency of hypoglycemia focused on the importance of carbohydrate consistency and prioritizing reduction of fat intake when reducing calories to prevent weight gain and teaching tailored adjustment of insulin dose for activities of various intensities and durations based on blood glucose monitoring results [48].

The Academy of Nutrition and Dietetics has established evidenced-based diabetes MNT for patients with type 1 diabetes [56]. In the randomized field test, specific guidelines for nutrition counseling were used by dietitians with 24 patients, and results were compared with those of 30 patients receiving "usual counseling" as the control treatment condition. The mean A1c in the guidelines-treated patient group was significantly reduced compared with the control group (1.0% vs 0.3%) [57].

The Dose Adjustment for Normal Eating study demonstrated improvements in A1c, quality of life, psychological well-being, and satisfaction with treatment in individuals with type 1 diabetes, who learned to use glucose testing to better match insulin to carbohydrate intake despite an increase in the number of daily glucose tests and insulin injections [58]. The quality-of-life improvements were maintained at the 4-year follow-up, although the glycemic control improvements were maintained to a lesser extent [59].

2 Type 2 Diabetes

The United Kingdom Prospective Diabetes Study (UKPDS) examined the benefit of metabolic control (glucose and blood pressure) in patients newly diagnosed with type 2 diabetes [41,60−64]. Fundamentally, the UKPDS confirmed that the findings of the DCCT also apply to type 2 diabetes. There was a reduction in macrovascular and microvascular complications, and the best results were obtained in those individuals who achieved both glucose and blood pressure control. Similarly, there was a clear dose−response relationship between metabolic and blood pressure control and the risk of diabetes complications.

The Lifestyle Over and Above Drugs in Diabetes randomized, controlled trial in New Zealand examined the impact of intensive nutritional counseling over a period of 6 months in patients with type 2 diabetes ($n = 93$). Significant reductions in the intervention group were observed in A1c, weight, BMI, and waist circumference compared to the control group [65]. MNT for treatment of type 2 diabetes focused on weight loss and physical activity to improve insulin sensitivity and metabolic control of glucose, lipids, and blood pressure.

The ADA and the EASD position statement recommends metformin as the optimal drug for monotherapy of diabetes and then outlines the properties of available glucose-lowering agents in the United States and Europe (Table 31.3) that may guide individualized treatment options to achieve glycemic targets [44,45]. Many

patients with type 2 diabetes take five or more medications to achieve blood glucose, blood pressure, and cholesterol goals, as well as low-dose aspirin. The impact of weight loss is most dramatically demonstrated by bariatric surgery, although the effects of bariatric surgery on diabetes appear to be largely independent of weight loss and may be due to changes in hormonal metabolism [66].

The Look-AHEAD (Action for Health in Diabetes) study, a multicenter, randomized, controlled trial of an intensive lifestyle intervention in 5145 patients with type 2 diabetes, found that a weight loss of 8.6% was associated with improved diabetes control, as indicated by reductions in diabetes medication use and A1c levels [67]. Four-year results of the Look-AHEAD study indicate that significant improvements in weight loss, fitness, and A1c observed in the intensive lifestyle intervention group compared to the control group were sustained [68]. By study end, the differences in CVD risk factors had diminished, with A1c and blood pressure showing the most sustained improvements. The Look-AHEAD lifestyle intervention was adapted from the DPP intervention to meet the needs of people with diabetes and incorporated the use of meal replacements to provide structure and enhance weight loss results [69]. These lifestyle interventions are currently being translated into clinical practice settings for primary care patients in more cost-efficient ways with comparable effectiveness in terms of weight loss, A1c, and reductions in medications and associated costs [70].

The Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) study found that metformin alone achieved durable glycemic control (A1c < 8%) in approximately half of the subjects, suggesting that many youth with type 2 diabetes are likely to require combination treatment within a few years of diagnosis [71–75]. The participants in this clinical trial were reported to consume unhealthful diets and be unfit [72,73]; dyslipidemia, hypertension, microalbuminuria, and chronic inflammation were common and appeared to worsen over time in these youth regardless of diabetes treatment [74,75]. Youth with type 2 diabetes and their families need to prioritize lifestyle modifications such as eating a healthful diet, maintaining a healthy weight, and exercising regularly. Nutrition and lifestyle interventions that are culturally appropriate and sensitive to family resources are needed to improve the diets and lifestyle-related health outcomes of these youth [71].

IV APPROACHES TO TREAT COMORBIDITIES AND REDUCE COMPLICATIONS

The rates of CVD and related complications have improved significantly over the past decade and morbidity and mortality have decreased [43]. Diabetes is also the leading cause of kidney failure, nontraumatic amputations, and new cases of blindness for U.S. adults. The Action to Control Cardiovascular Risk in Diabetes study was a randomized, controlled trial that examined the effects of intensive glycemic control (target A1c < 6%) versus standard glycemic control (target A1C between 7% and 7.9%) in 10,251 patients with extant diabetes on CVD outcomes. The treatment arm receiving intensive control of blood pressure and lipids did not have a significant improvement over controls [76]. Results from two other large, randomized, controlled trials—the ADVANCE study and the Veterans Affairs Diabetes Trial—also found no significant reduction in CVD risk with intensive glycemic control [38,77]. The results of these three trials indicate that intensive treatment may not provide any additional benefit, at least in the time period of treatment and populations observed in these studies [78].

The Look-AHEAD study also examined the effect of achieving and maintaining weight loss on cardiovascular risk reduction. One-year results of the intervention indicated that the clinically significant weight loss (8.6%) was associated with reductions in CVD risk factors as indicated by blood pressure, triglycerides, HDL cholesterol, and urine albumin-to-creatinine ratio [67]. Reductions in C-reactive protein have also been observed [79]. Four-year results of the study indicate that the improvements in systolic blood pressure and HDL cholesterol levels observed in the intensive lifestyle intervention group compared to the control group were sustained [68]. After a median follow-up of 9.6 years, the Look-AHEAD trial was stopped early based on a futility analysis [80]. There were no significant differences found between the lifestyle intervention and the diabetes support and education treatment groups in the hazard rates for the primary composite CVD outcomes (fatal and nonfatal heart attacks and strokes, hospitalized angina). The overall cardiovascular event rate was low in both groups, with the lifestyle intervention group achieving greater weight loss and activity levels and the diabetes support and education group receiving greater amounts of medications for A1c, blood pressure, and lipid management [80]. At study end, the lifestyle intervention group had sustained a weight loss of 6% versus 3.5% weight loss in the diabetes support and education group. Moreover, the lifestyle intervention group achieved many other clinical and psychological benefits compared with the diabetes support and education group. They achieved improved outcomes for A1c, blood pressure, triglycerides, C-reactive protein, self-reported retinopathy, nephropathy, diabetes remission rates, sleep apnea, fatty liver disease, sexual dysfunction, urinary incontinence, physical function, knee pain, quality of life, depression and body image dissatisfaction and

reductions in number of medications, medication costs, and hospitalizations. These results have important implications for clinical practice [81].

V NUTRIENT INTAKE CONSIDERATIONS

The ADA and other diabetes organizations address nutrient intake in their nutritional recommendations [9,82,83]. The guidelines are similar; therefore, this section focuses on ADA [9] and highlights a few additional recommendations developed by the EASD [82,83].

A Protein

The ADA recommends basing protein intake for individuals with all classes of diabetes and normal renal function largely on the review by the Institute of Medicine to establish recommendations for the general public with 15−20% of energy intake from protein [9]. The EASD recommends 10−20% of energy intake from protein without established nephropathy [82]. Individuals who may need more than 20% of energy intake from protein include those in a catabolic state, those with growth needs (children, adolescents, and pregnant women), and individuals on very low energy diets to achieve weight loss.

B Fat

Intake of fat can blunt and extend postprandial glycemic excursions. An evaluation of the intensively treated DCCT patients revealed that a higher intake of fat and saturated fat and lower intake of carbohydrate were associated with worse glycemic control, independent of exercise and BMI [84]. The ADA recommends that all individuals with diabetes limit their saturated fat to less than 7% of total energy, minimize *trans*-fat intake [9]. In addition to these recommendations, the ADA also recommends consumption of two or more servings of fish per week in order to increase intake of *n*-3 fatty acids, which may contribute to improved cardiometabolic risk factor outcomes [9]. Similar to the ADA, the EASD recommends limiting saturated and *trans*-fat intakes and consuming two or three servings of fish per week [9,82].

C Carbohydrates

Dietary carbohydrate is the major determinant of postprandial glucose concentration and is therefore integral to glycemic control. The ADA recognizes total carbohydrate as the major determinant of postprandial glucose concentration, with the type of carbohydrate as an additional determinant [9]. Although fructose is associated with a reduction in postprandial hyperglycemia when it is substituted for sucrose or starch, the long-term consequences of high fructose intake on plasma lipids and other diabetic complications is unknown. Based on a 2009 meta-analysis of 16 trials reporting that the effects of fructose intake on blood lipids in individuals with type 2 diabetes were mixed [9,85], both the ADA and EASD make no specific recommendation regarding the use of fructose [9,82,83]. The ADA's nutrition recommendations indicate that glycemic control is not contingent on restricting sucrose and suggest that the decision about sugar consumption should be based on overall nutrition considerations [9]. Nonetheless, consumption of large quantities of sugars (e.g., high-fructose corn syrup in soft drinks and other beverages) can make a substantial contribution to the intake of excess calories [86].

The concept of glycemic indexing of food was developed to compare the effects of the quality of carbohydrate while keeping the amount of carbohydrate standardized. The estimated glycemic load of foods, meals, and dietary patterns is calculated by multiplying the glycemic index by the amount of carbohydrate in each food and then totaling for all of the foods in a meal or dietary pattern. The role of the glycemic index and/or glycemic load lacks consensus recommendations, although modifying the type as well as the amount of carbohydrate can improve glycemic control [9]. A randomized trial conducted in children with type 1 diabetes achieved better A1c and quality-of-life outcomes with a flexible low-glycemic index diet than with a measured carbohydrate exchange diet [87]. Randomized controlled trials in individuals with type 2 diabetes have achieved mixed results: A 6-month trial found a moderate decrease in A1c [88], whereas a 1-year trial found no improvement in A1c [89]. A third trial found a decrease in A1c in both the low-glycemic diet group and the ADA diet education group at 1 year [90]. Meta-analysis results have also been mixed; a review by Brand-Miller et al. [91] found significant decreases in A1c levels while on low-glycemic diets, whereas Anderson et al. [92] found no significant difference in A1c levels between a low- and high-glycemic diet. Taking this evidence into account, the ADA and the EASD recognize that considering the glycemic index of carbohydrates may provide additional benefit over considering total carbohydrate alone, provided that the overall attributes of the carbohydrate are taken into account [9,82,83]. More recently, research on an 800-person cohort without diabetes revealed that people eating identical meals present high variability in postmeal blood glucose response and that use of an algorithm that integrates blood parameters, dietary habits, anthropometrics, physical activity, and gut microbiome features enabled accurate glucose response prediction [93]. Future research will further elucidate the utility of this approach over longer periods of time. In the meantime, both organizations recommend dietary patterns that include carbohydrates from

whole grain cereals, fruits, vegetables, legumes, and low-fat milk [9,82].

Findings from several randomized, controlled trials [94–96] indicate that fiber supplements (additional 4–19 g/day) do not improve glycemia or CVD risk factors, and therefore the recommended dietary intake for the general public is also recommended for people with diabetes [9]. In essence, given the many factors that can affect glucose metabolism, including those beyond nutrition per se (e.g., medicines and activity), it is often problematic to predict the exact plasma glucose response to specific carbohydrate-containing foods. Certainly, blood glucose self-monitoring and experience can help predict the glycemic effects of food products. Furthermore, a variety of methods can be used to estimate the nutrient content of meals, including carbohydrate counting, the exchange system, and experience. With emerging evidence of the relations between postprandial glycemia and CVD [97], postprandial glucose levels are of increasing importance.

Fructose, mannitol, and sorbitol are often substituted for sucrose in "sugar-free" products. In experimental studies, these products can shift the balance from oxidation of fatty acids to esterification of fatty acids in the liver, which can in turn increase VLDL synthesis [56]. Although the effects on serum lipids are inconsistent, susceptible individuals may have a worsening of dyslipidemia. These sweeteners appear to offer no documented advantage in the management of diabetes over other carbohydrate sources.

Labeling of food products with regard to carbohydrate composition can be confusing. Food products may list the "net" or "impact" carbohydrate on the front of the label, a value considerably lower than the "total" carbohydrate listed on the nutrient facts panel. Fiber or fiber plus the sugar alcohols are usually subtracted to obtain the net or impact carbohydrate value, but there is no standardization [98]. If patients with diabetes use these products, monitoring is needed to determine the effects on blood glucose. Nonnutritive sweeteners, which are considered high-intensity sweeteners, are widely used as a replacement of various types of sugar in food and beverage products. An evidence-based review by the Academy of Nutrition and Dietetics suggests that all approved intense sweeteners, also known as noncaloric and nonnutritive sweeteners (aspartame, saccharin, acesulfame K, sucrolose, and stevia), have no adverse effects on diabetes management [56].

Alcohol can inhibit hepatic glucose production, resulting in hypoglycemia if consumed without food when the diabetes treatment regimen includes insulin or sulfonylureas [56]. Conversely, consuming large amounts of alcohol can increase blood glucose levels during severe insulin deficiency. A systemic review found that alcohol intake, diabetes, hypertension, and hypertriglyceridemia are associated with increased risk of developing gout [99]. Alcohol intake can exacerbate pancreatitis, severe hypertriglyceridemia, severe neuropathy, myocardiopathy, or renal failure, which are comorbidities associated with diabetes.

The Study to Help Improve Early Evaluation and Management of Risk Factors Leading to Diabetes findings [100] have demonstrated that for people with diabetes there is a significant gap between knowledge of nutrition and lifestyle recommendations (what to do) and how to incorporate these recommendations into lifestyle routines. MNT is the process by which these evidence-based nutrition recommendations are translated into a plan that individuals can follow based on a thorough assessment of each person's lifestyle, capabilities, and motivation to change. The tools and techniques to facilitate behavior change are discussed in another chapter.

VI COLLABORATIVE EFFORTS FOR DIABETES PREVENTION AND TREATMENT

In the United States, much effort within the voluntary, professional, academic, and private sectors is directed at addressing the challenges of diabetes. The National Diabetes Education Program (NDEP), a partnership of the National Institutes of Health and the CDC, serves as a "coordinating entity" among public and private organizations (http://www.cdc.gov/diabetes/ndep/index.html). The NDEP developed the Guiding Principles for the Care of People with or at Risk for Diabetes in collaboration with several partner organizations. The Guiding Principles recommend providing patient-centered care and shared decision making, which is achieved by "eliciting patient perspectives and presenting options and information so patients can participate more actively in care." Use of the medical home model is suggested to provide "accessible, continuous, comprehensive, and coordinated care that is delivered by a health care team in the context of family and community" [101]. Guiding Principles for Diabetes educational materials and other resources are available from the NDEP and some of its partners from their websites (Table 31.4).

In addition, the CDC's Division of Diabetes Translation is addressing community infrastructure and environmental issues to reduce the burden of diabetes, including public health surveillance systems for diabetes, applied translational research, state-based diabetes control programs, and public information (see http://www.cdc.gov/diabetes/about/). The National Institutes of Health has also expanded the focus of research to address how environmental factors and community infrastructure are related to obesity and the risk of diabetes and other chronic

TABLE 31.4 Information Sources for Diabetes

Agency for Healthcare Research and Quality: http://www.ahrq.gov/professionals/clinicians-providers/ehclibrary/diabetes/index.html
American Association of Diabetes Educators: http://www.diabeteseducator.org
American Diabetes Association: http://www.diabetes.org
Centers for Disease Control and Prevention: http://www.cdc.gov/diabetes, http://www.cdc.gov/diabetes/home/index.html, http://www.cdc.gov/nchs
Centers for Medicare and Medicaid Services: https://www.cms.gov/Outreach-and-Education/Medicare-Learning-Network-MLN/MLNMattersArticles/downloads/se0738.pdf
Health Resources and Services Administration: http://www.hrsa.gov/quality/toolbox/measures/diabetes/
International Diabetes Federation: http://www.idf.org/
Juvenile Diabetes Research Foundation International: http://www.jdrf.org
National Diabetes Education Program, a joint program of the National Institutes of Health and the Centers for Disease Control and Prevention: http://www.yourdiabetesinfo.org
National Diabetes Information Clearinghouse: http://www.diabetes.niddk.nih.gov
National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health: http://www.niddk.nih.gov
U.S. Department of Health and Human Services, Office of Minority Health: http://minorityhealth.hhs.gov
U.S. Department of Veterans Affairs: http://www.healthquality.va.gov
U.S. Food and Drug Administration: http://www.fda.gov

diseases through the National Institute of Diabetes, Digestive and Kidney Disease research program. See http://www.niddk.nih.gov/research-funding/research-programs/Pages/clinical-research-type-2-diabetes.aspx.

VII CONCLUSION

The long-term goal of diabetes prevention and treatment is restoring metabolism as close to normal as possible in order to reduce the morbidity and mortality associated with diabetes. The focus for all classes of diabetes is on reducing cardiovascular risk factors such as hypertension and dyslipidemia. The distribution of macronutrient intake may vary based on a number of factors, including matching insulin to lifestyle in type 1 diabetes and weight loss in type 2 diabetes.

MNT is based on individual assessment and development of a personalized tailored evidence-based treatment plan. An RD who consults with the health care team and assesses the patient's needs is well positioned to develop a tailored treatment plan that considers overall health needs and individual capabilities with the goal of ameliorating the metabolic effects of diabetes and its complications.

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Chapter 32

Nutritional Management for Gestational Diabetes

Alyce M. Thomas[1] and Maria Duarte-Gardea[2]

[1]St. Joseph's Regional Medical Center, Paterson, NJ, United States, [2]The University of Texas at El Paso, El Paso, TX, United States

I INTRODUCTION

Gestational diabetes mellitus (GDM) is diabetes diagnosed in the second or third trimester of pregnancy that is not clearly either type 1 diabetes (T1DM) or type 2 diabetes (T2DM) [1]. This is how the American Diabetes Association (ADA) defines GDM in its 2016 Standards of Medical Care and it represents a shift in the previous definition. Prior to 2016, GDM was defined for many years as carbohydrate intolerance of variable degree with onset or first recognition during pregnancy and included all women regardless of whether the condition may have predated the pregnancy. Now, according to the ADA, diabetes diagnosed in the first trimester is considered undiagnosed T2DM and the treatment protocol is the same as for women with T2DM.

However, other organizations have not adopted this definition. The World Health Organization (WHO) defines GDM as hyperglycemia that is first recognized during pregnancy [2]. GDM is defined by the American College of Obstetricians and Gynecologists (ACOG) as a condition in women who have carbohydrate intolerance with onset or recognition during pregnancy [3].

GDM is associated with complications during and after pregnancy. The woman is at risk of developing GDM in subsequent pregnancies and/or T2DM in the future. Exposure to maternal hyperglycemia in utero conveys a risk for obesity and T2DM in the offspring. The key to prevention or decreasing the risk of complications is to achieve and maintain optimal glycemic control during the pregnancy. The management of GDM includes medical nutrition therapy (MNT), physical activity, and, if indicated, pharmacological therapy [4]. An interdisciplinary health care team, which includes endocrinologists, obstetricians, registered dietitians, diabetes educators, nurses, social workers, and other professionals, works in collaboration with the common goal of normoglycemia from diagnosis to postpartum.

The benefits of lifestyle modifications go beyond the immediate postpartum period. It may represent an opportunity for the registered dietitian and other health care providers to impact lifestyle patterns aimed toward establishing future habits that will benefit the woman and her family.

II PREVALENCE

Almost 4 million babies were born in the United States in 2014 [5]. Although the true prevalence of GDM is unknown, it is estimated to affect 1−14% of pregnancies in the United States annually [6]. In a study using the Pregnancy Risk Assessment Monitoring System, the prevalence was 9.2% [6], which could mean the number of pregnancies affected by GDM is greater than 350,000 per year. This variation in the prevalence depends on the population studied and the diagnostic tests used [7]. In populations with a higher prevalence of obesity and T2DM, the prevalence of GDM is also higher [8,9]. This includes Native Americans, Asians, Hispanics, and African American women [10].

A cohort study in Colorado that examined the trends in GDM prevalence among women with diverse ethnic backgrounds reported an increase from 1.7% to 3.1% among non-Hispanic whites; from 2.8% to 5.4% in Hispanics, and from 2.9% to 5.4% in African Americans [11]. A similar trend has been reported for Canadian aboriginal populations. The prevalence in the inland communities was twice as high as in the coastal communities (18.0% vs 9.3%, $p = 0.002$) [12].

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TABLE 32.1 Risk Factors for Testing for Diabetes in Pregnant Women at Their Initial Prenatal Visit

BMI ≥ 25 kg/m^2 or ≥23 kg/m^2 in Asian Americans and with additional risk factors:

- Physical inactivity
- First-degree relative with diabetes
- High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
- Previous delivery of infant whose birth weight >9 lb or previously diagnosed with GDM
- Hypertension (≥140/90 mm Hg on therapy for hypertension)
- HDL-cholesterol level <35 mg/dL (0.90 mmol/L) and/or triglyceride level >250 mg/dL (2.82 mmol/L)
- Polycystic ovary syndrome
- A1C ≥ 5.7%
- Other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)
- History of cardiovascular disease

Source: From American Diabetes Association, Standards of Medical Care—2016. Classification and diagnosis of diabetes, Diabetes Care 39 (Suppl. 1) (2016) S13–S22.

TABLE 32.2 Maternal and Fetal Complications Associated With GDM

Maternal	Fetal
• Hypertension	• Macrosomia
• Preeclampsia	• Neonatal hypoglycemia
• Premature delivery	• Neonatal hypocalcemia
• Cesarean delivery	• Hyperbilirubinemia
• GDM in subsequent pregnancies[a]	• Polycythemia
• Type 2 diabetes[a]	• Respiratory distress syndrome
• Cardiovascular disease[a]	• Stillbirth
• Metabolic syndrome[a]	• Shoulder dystocia

[a]*Postpregnancy complications.*

Source: From E.A. Reece, The fetal and maternal consequences of gestational diabetes mellitus, J. Matern. Fetal Neonate Med. 23 (2010) 199–203; T.L. Setji, A.J. Brown, M.N. Feinglos, Gestational diabetes mellitus, Clin. Diabet. 23 (2005) 17–24.

III RISK FACTORS

Risk factors associated with GDM also vary among organizations. Women were previously categorized as being at low, average, or high risk for developing GDM. Low-risk women were not universally screened for GDM because it was not considered cost-effective since they represented only 10% of the pregnant population. However, according to the ACOG, if traditional historical factors are used (family or personal history of diabetes, previous adverse pregnancy outcome, glycosuria, and obesity) to identify GDM, approximately 50% of women with GDM will be missed [3]. ADA identifies women at risk for developing GDM in Table 32.1.

IV COMPLICATIONS ASSOCIATED WITH GDM

Although complications associated with GDM are generally not as severe when compared to pregnancies with preexisting diabetes, perinatal morbidity and even mortality may occur with uncontrolled glycemic levels (Table 32.2). Maternal risks associated with gestational diabetes include hypertension, and increased risk of operative and preterm deliveries. In the infant, complications include macrosomia, neonatal hypoglycemia, neonatal hypocalcemia, neonatal hyperbilirubinemia, and polycythemia. Shoulder dystocia, clavicular fracture, brachial palsy, and respiratory distress syndrome are associated with GDM more so than in infants born to women with normoglycemia. Macrosomia, which is defined as a birth weight greater than 4000 g or 4500 g, is the most common complication associated with GDM and occurs in approximately 20% of pregnancies in undiagnosed or untreated GDM [13]. Pedersen hypothesized that maternal hyperglycemia results in fetal hyperinsulinemia, lipogenesis, glycogen, and protein synthesis and subsequent macrosomia [14]. In a prospective study that included 115 untreated women with borderline GDM by the broader criteria of Carpenter and Coustan, the untreated borderline GDM group had increased rates of macrosomia (28.7% vs 13.7%, $p < 0.001$) and cesarean delivery (29.6% vs 20.2%, $p = 0.02$) when compared with normoglycemic controls [15].

The effect of complications that develop during pregnancy may extend beyond delivery. Women with a history of GDM are at increased risk of developing the condition in a subsequent pregnancy or T2DM within 10 years. Her infant is at a higher risk of developing obesity and T2DM later in life.

V SCREENING AND DIAGNOSIS

Currently, there is no gold standard for the screening or diagnosis of GDM. This inconsistency is the result of the lack of data that shows adverse outcomes at various levels of hyperglycemia. O'Sullivan and Mahan established the first diagnostic testing for GDM using a 3-hour oral glucose tolerance test (OGTT) and whole blood [16]. The National Diabetes Data Group (NDDG) proposed using plasma blood glucose and defined GDM as two or more values higher than the thresholds [17]. Carpenter and Coustan proposed using diagnostic criteria which was lower than those of the NDDG [18]. This diagnostic criteria also used a two-step approach, with a 50-g, 1-hour glucose challenge test to screen for

diabetes as the first step. Depending on the results of the screening (usually >135 mg/dL or 7.5 mmol/L), the 100-g, 3-hour OGTT was performed to diagnose GDM. This two-step process of screening and diagnosing GDM is still widely used by many obstetricians.

In 2008, a large multinational study of approximately 23,000 pregnant women, known as HAPO (Hyperglycemia and Adverse Pregnancy Outcomes), demonstrated adverse perinatal outcomes (infant birth weight greater than the 90th percentile, cord-blood serum C-peptide, primary cesarean section, and neonatal hypoglycemia) at levels that were considered normal in pregnancy [19]. Based on the HAPO data, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) proposed new cutoffs for the diagnostic criteria for GDM [20]. These criteria which use the 75-g, 2-hour one-step approach and have been adopted by the ADA are not universally accepted (see Box 32.1). In 2013, the National Institutes of Health's Consensus Panel on Diagnosing GDM recommended the continuation of the two-step approach because of the lack of evidence which showed the 2-hour OGTT improved perinatal outcomes [21]. The two-step approach is supported by the ACOG. (See Table 32.3 for the diagnostic criteria of various organizations).

BOX 32.1 Oral Glucose Tolerance Test

One-Step Approach	Two-Step Approach
	Oral Glucose Challenge Test (GCT) • 50-g glucose solution given at any time of day • If results <135 or 140 mg/dL 1 h later, administer 3-h OGTT 3 days later
Oral Glucose Challenge Test (OGTT) • 3 days before—normal carbohydrate load of at least 150 g • fast at least 8 h before test • 75-g glucose solution given • blood drawn at fasting, and 1 and 2 h • during test—no eating, smoking, drinking (except water), or walking (remain seated) • diagnosis made if one value is abnormal	Oral Glucose Challenge Test (OGTT) • 3 days before—normal carbohydrate load of at least 150 g • fast at least 8 h before test • 100-g glucose solution given • blood drawn at fasting, and 1, 2, and 3 h • during test—no eating, smoking, drinking (except water), or walking (remain seated) • diagnosis made if two values are abnormal

VI WEIGHT GAIN IN PREGNANCY

The current weight gain guidelines from the Institute of Medicine are based on women's prepregnancy BMI. Table 32.4 shows the guidelines for total weight gain for each BMI category and the weekly weight gain for the second and third trimesters [22]. Women should be advised to gain according to their BMI category. Inadequate weight gain is associated with low birth weight and small-for-gestational-age infants. Excessive weight gain may lead to macrosomia in the infant, cesarean delivery, and postpartum weight retention. A prenatal weight gain grid can be used to monitor and evaluate the amount of weight gain. (See Table 32.5 for an example of a prenatal weight gain grid for normal weight women.)

VII MONITORING IN PREGNANCY

Monitoring during GDM provides the woman and her health care team with the necessary tools to assist her with diabetes management. These tools include self–blood glucose monitoring, ketone monitoring, and glycosylated hemoglobin (A1C).

A Blood Glucose Monitoring

The use of self-monitoring blood glucose (SMBG) allows the medical team to objectively evaluate and, if necessary, adjust the meal plan or medication. The ADA and ACOG recommend that women with GDM monitor their blood glucose levels four times daily—fasting and after meals [3,4]. However, the timing of postmeal monitoring has been debated for many years. According to the ACOG Practice Bulletin on GDM, no research has demonstrated the superiority of either 1- or 2-hour postmeal testing [3]. Other studies using continuous glucose monitoring (CGM) to determine the peak glucose elevation in pregnant woman showed that the peak time was 60–90 minutes from the beginning of a meal [23–25]. Whether monitoring is performed 1 or 2 hours after eating, it should be from the beginning, and not the end, of the meal. The frequency of monitoring may be reduced from daily to every third or fourth day once control of blood glucose is established. The target blood glucose levels in pregnancy are found in Table 32.6.

B Urinary Ketone Testing in GDM

While blood glucose monitoring is generally recommended in the management of GDM, ketone testing is not. Pregnancy is considered a ketogenic state and ketones may be found in the urine in the nondiabetic pregnant woman. However, the presence of ketones may suggest starvation ketosis as the result of inadequate energy intake. One early

TABLE 32.3 Diagnostic Criteria for GDM for Various Organizations

Organization	Glucose Challenge Test (50 g)	Fasting Blood Glucose	1-Hour Blood Glucose	2-Hour Blood Glucose	3-Hour Blood Glucose
National Diabetes Data Group[a]		105 mg/dL (5.8 mmol/L)	190 mg/dL (10.5 mmol/L)	165 mg/dL (9.2 mmol/L)	145 mg/dL (8.0 mmol/L)
ACOG[a]	<135 or 140 mg/dL	≥95 mg/dL (5.3 mmol/L)	≥180 mg/dL (10.0 mmol/L)	≥155 mg/dL (8.6 mmol/L)	≥140 mg/dL (7.8 mmol/L)
IADSPG, ADA[a,b]	–	≥92 mg/dL (5.1 mmol/L)	≥180 mg/dL (10.0 mmol/L)	≥153 mg/dL (8.5 mmol/L)	–
WHO[b]	–	92–125 mg/dL (5.1–6.9 mmol/L)	>180 mg/dL (≥10.0 mmol/L)	153–199 mg/dL (8.5–11.0 mmol/L)	–

[a] *100 g, 3-hour OGTT.*
[b] *– 75 g, 1-hour OGTT.*
ACOG, American College of Obstetricians and Gynecologists; ADA, American Diabetes Association; IADPSG, International Association of Diabetes and Pregnancy Study Groups; WHO, World Health Organization.

TABLE 32.4 Institute of Medicine Weight Gain in Pregnancy Guidelines

Prepregnancy BMI	Recommended Weight Gain	Rate of Gain/Week (2nd and 3rd trimesters)	Recommended Total Weight Gain (Twin Gestation)
Underweight (<18.6)	28–40 lb (12.7–18.2 kg)	1½ lb (0.7 kg)	
Normal weight (18.6–24.9)	25–35 lb (11.2–15.9 kg)	1 lb (0.5 kg)	37–54 lb (16.8–24.4 kg)
Overweight (25.0–29.9)	15–25 lb (6.8–11.3 kg)	2/3 lb (0.3 kg)	31–50 lb (14.1–22.7 kg)
Obese (>30.0)	11–20 lb (4.5–9.0 kg)	Individualize 1/2 lb (0.25 kg)	25–42 lb (11.3–19.1 kg)

Source: From National Academy of Sciences, Weight Gain During Pregnancy: Reexamining the Guidelines, National Academy Press, Washington, DC, 2009.

study had indicated a possible link between ketonemia and decreased intelligence in the offspring [26]. Neither the ADA nor the ACOG include ketone testing for women with GDM in the Standards of Medical Care or Practice Bulletin on GDM, respectively [3,4]. However, women with GDM should avoid controlling their blood glucose levels by consuming insufficient calories.

C Hemoglobin A1C

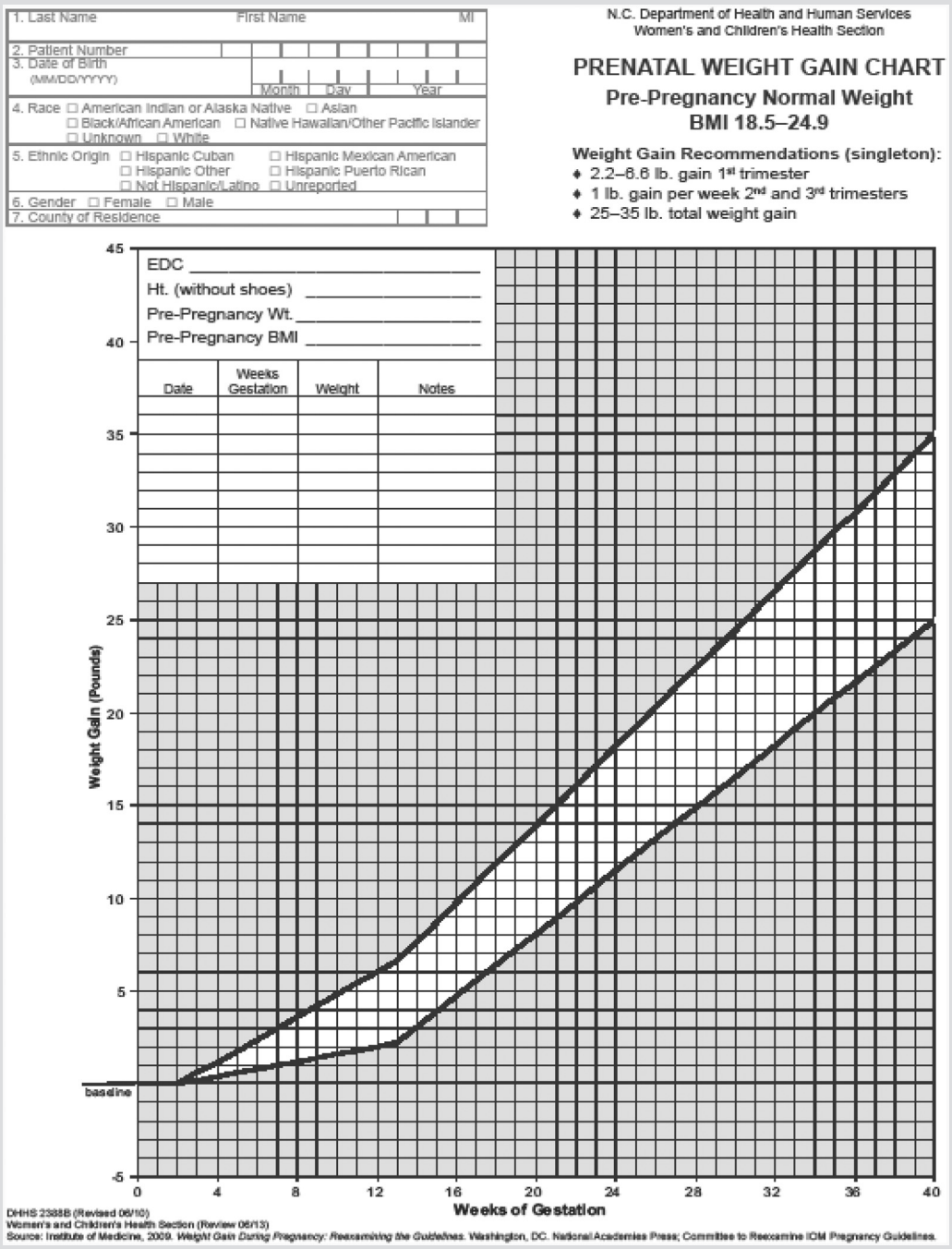
The target of A1C in pregnant women with preexisting diabetes is 6–6.5% (42–49 mmol/mol) [4]. There is currently no recommendation to monitor the A1C in women with GDM.

VIII NUTRITION MANAGEMENT

MNT for GDM primarily involves a carbohydrate-controlled meal plan that promotes optimal nutrition for maternal and fetal health with adequate energy for appropriate gestational weight gain, achievement and maintenance of normoglycemia, and absence of ketosis. Due to the continuous fetal draw of glucose from the mother, maintaining consistency of times and amounts of food eaten are important to avoid hypoglycemia. During pregnancy, the distribution of energy and carbohydrate intake should be individualized and based on the woman's food preferences and plasma glucose responses. Plasma glucose monitoring and daily food records provide valuable information for insulin and meal plan adjustments [27]. The estimated energy requirements (EERs) during the first trimester are the same as those for a nonpregnant woman with an additional 340 calories during the second and 452 calories during the third trimesters [28]:

1st trimester: adult EER for women (no calorie increase);
2nd trimester: adult EER for women + 160 kcal (8 kcal*/week × 20 week) + 180 kcal**; and
3rd trimester: adult EER for women + 272 kcal (8 kcal*/week × 34 week)+180 kcal**.
*8 kcal/week = estimated change in the total energy expenditure in pregnancy.
**180 = mean energy deposition during pregnancy.

TABLE 32.5 Prenatal Weight Gain Grid



Source: Downloaded from N.C. Department of Health and Human Services Women's and Children's Health Section. http://www.nutritionnc.com/wic/pdf/NormalWeightSingletonGestationWeightGainChart.pdf.

TABLE 32.6 Target Plasma Glucose in Gestational Diabetes Pregnancy

Time	American Diabetes Association and American College of Obstetricians and Gynecologists	CDAPP	International Diabetes Federation
Fasting	≤95 mg/dL (5.3 mmol/L)	≤ 60–89 mg/dL (3.3–5.0 mmol/L)	<90 mg/dL (5.5 mmol/L)
1 hour	≤130 mg/dL (7.2 mmol/L)	100–120 mg/dL (5.6–6.7 mmol/L)	<140 mg/dL (7.7 mmol/L)
2 hours	≤120 mg/dL (6.7 mmol/L)		<120 mg/dL (6.6 mmol/L)

CDAPP, California Diabetes and Pregnancy Sweet Success.
Source: From International Diabetes Federation IDF GDM Model of Care Implementation Protocol Guidelines for Healthcare Professionals. http://www.idf.org/sites/default/files/attachments/GDM-model-of-care-2015.pdf (accessed 01.06.16).

The amount and distribution of carbohydrate should be based on clinical outcome measures including hunger, plasma glucose levels, weight gain, and ketone levels. To ensure provision of glucose to the fetal brain (approximately 33 g/day) a minimum amount of 175 g/day of carbohydrates should be provided [28]. Carbohydrate should be distributed throughout the day in three small- to moderate-sized meals and two to four snacks. An evening snack may be needed to prevent accelerated ketosis overnight. Carbohydrate is generally less well tolerated at breakfast than at other meals [29].

A Carbohydrates

Carbohydrate is the primary nutrient affecting postprandial glucose levels, and postprandial blood glucose concentration is the one that has the most important role in macrosomia [30]. The conventional diet approach to GDM advocates for carbohydrate restriction, resulting in higher fat content, which is also a substrate for fetal fat deposit and associated with maternal insulin resistance. Consequently, there is no consensus about the optimal GDM diet. The traditional approach to diet therapy in GDM has been carbohydrate restriction (30–40% energy from total calories), with the goal of reducing postprandial glucose, in order to mitigate glucose-mediated fetal macrosomia [3,31].

However, in a randomized controlled trial of 152 women with GDM that were assigned to follow a diet low (40%) or high (55%) of total energy from carbohydrates (CHO), no difference was found in insulin use between the groups. In addition, no differences were found in the obstetric and perinatal outcomes between the treatment groups (e.g., ketonuria, hypertension, cesarean sections) [32]. The blood glucose levels of women with GDM were significantly lower after lunch and dinner when they were exposed to a high-carbohydrate diet. In another study that compared two different carbohydrate levels in women with GDM, 30 Caucasian women were randomly assigned to follow either a low- or a high-carbohydrate diet (45% and 65% of energy, respectively). The blood glucose levels of women who followed the low CHO diet were significantly lower after breakfast (102 ± 16 vs 94 ± 11 mg/dL), lunch (105 ± 12 vs 99 ± 9 mg/dL), and dinner (112 ± 16 vs 103 ± 13 mg/dL) ($p < 0.05$). Women following the high carbohydrate diet also experienced a significant decrease in glycemia after lunch (106 ± 15 vs 96 ± 7 mg/dL) and dinner (107 ± 12 vs 97 ± 7 mg/dL) ($p < 0.05$); however, their glucose concentration after breakfast did not change. It was concluded that high and low carbohydrate diets are effective and safe; however, a low-carbohydrate diet should be recommended to women who experience high glucose levels after breakfast [33].

B Dietary Fat

Fat content in the diet of a pregnant woman usually ranges from 30% to 40% of total calories; however, large amounts of fat beyond total caloric needs should be avoided to prevent excessive weight gain, which can result in further insulin resistance. The acceptable macronutrient distribution range of fat is 20–35% of total energy, the same as for nonpregnant women [28]. The impact of increasing polyunsaturated fatty acid intake on blood glucose, lipid metabolism, and pregnancy outcomes of women with GDM was assessed in a randomized controlled study that included 84 pregnant Chinese women. Women were randomly divided into two groups: an intervention group (prescribed a daily oil-rich diet of 45–50 g sunflower oil, 50–54% carbohydrates, 31–35% fat) and a control group (low-oil diet of 20 g sunflower oil, 55–60% carbohydrates, 25–30% fat). In both the intervention and the control groups, fasting blood glucose, 2-hour postprandial plasma glucose, and the insulin resistance index decreased significantly post-intervention ($p < 0.05$); the lipid changes and pregnancy outcomes did not differ significantly between the two groups ($p > 0.05$). It was concluded that an appropriate increase in polyunsaturated fatty acid intake benefits pregnant women with GDM as well as fetuses, as long as the diet therapy follows basic recommendations and total energy intake is strictly controlled [34].

In a randomized controlled study by Lauszus et al., the effects of a high monounsaturated fatty acid (MUFA) diet compared to recommended high-carbohydrate diet were studied in 27 women with GDM. After randomization women received either a high-carbohydrate diet (H-CHO): 50% carbohydrate, 20% protein, 30% fat [11% MUFA]) or a high-MUFA diet (H-MUFA): 46% carbohydrate, 16% protein, 37% fat [22% MUFA]) from the 33rd gestational week of pregnancy. Outcome measures were 24-hour ambulatory blood pressure, blood lipids, glycemic control, and insulin sensitivity estimated by an intravenous glucose tolerance test. Results indicated that the 24-hour diastolic blood pressure increased more in the H-CHO group than in the H-MUFA group ($p < 0.04$). The H-MUFA diet had no advantage to the H-CHO diet in ameliorating the decline of insulin sensitivity in the third term of pregnancy in GDM; however, the favorable effect on blood pressure by the MUFA diet might be used as a possible nonmedication treatment [35].

Furthermore, the effects of omega-3 fatty acid supplementation were studied in a randomized, double-blind, placebo-controlled study among 56 women with GDM. Subjects were randomly assigned to receive either 1000 mg omega-3 fatty acid supplements containing 180 mg eicosapentaenoic acid and 120 mg docosahexanoic acid ($n = 28$) or placebo ($n = 28$) for 6 weeks. Fasting blood samples were taken at baseline and after 6 weeks of intervention. A significant difference in changes in serum insulin levels (from baseline: -1.5 ± 7.5 vs $+3.5 \pm 8.5$ μIU/mL, $p = 0.02$) and homeostasis model assessment estimated insulin resistance (HOMA-IR) (-0.4 ± 2.1 vs $+1.1 \pm 2.4$, $p = 0.02$) was found when comparing the two groups. In addition, a significant reduction in serum high-sensitivity C-reactive protein (hs-CRP) levels was seen after omega-3 fatty acid supplementation when compared with the placebo (-236.3 ± 1541.9 vs 898.6 ± 2292.7 ng/mL, $p = 0.03$). The authors concluded that omega-3 fatty acid supplementation in GDM women had beneficial effects on insulin resistance. Plasma glucose, HOMA for beta-cells, quantitative insulin sensitivity check index, and lipid profiles were not affected [36].

The combination of omega-3 fatty acid supplementation with vitamin E was tested on glucose homeostasis parameters and lipid concentrations among women with GDM not taking oral hypoglycemic agents. In a randomized controlled trial, women were allocated to take either 1000 mg omega-3 fatty acids from flaxseed oil plus 400 IU vitamin E supplements ($n = 30$) or placebo ($n = 30$) for 6 weeks. Fasting blood samples were obtained at the beginning of the study and after 6-week intervention. Changes in fasting plasma glucose (-11.8 ± 11.0 vs $+1.5 \pm 11.9$ mg/dL, $p < 0.001$), serum insulin concentrations, HOMA-IR, HOMA of beta-cell function, and quantitative insulin sensitivity check index in the omega-3 fatty acids plus vitamin E group were significantly different from the changes in these indicators in the placebo group. Overall, it was demonstrated that omega-3 fatty acids and vitamin E cosupplementation in GDM women had beneficial effects not only on glucose homeostasis parameters but also in serum triglycerides, VLDL-cholesterol, and HDL-cholesterol concentrations. Supplementation of omega-3 fatty acids and vitamin E did not have an influence on total cholesterol and LDL-cholesterol levels [37].

C Protein

The diet of well-nourished women in the preconception period and throughout most of pregnancy has a significant effect on birth weight, and protein is the macronutrient with the greatest influence. Cuco et al. [38] demonstrated that in a protein and fat model, a 1-g increase in maternal protein intake during preconception and in the 10th, 26th, and 38th weeks of pregnancy led to a significant increase in birth weight of 7.8−11.4 g. Conversely, high intakes of protein and fat during pregnancy may impair development of the fetal pancreatic beta-cells and lead to insulin deficiency in the offspring [39]. In a case−control study of 2341 women with singleton pregnancies, three different levels of protein in the diet were associated with birth weight. Birth weight was 77 g lower ($p = -0.021$) in the low-protein group and 71 g lower ($p = 0.009$) in the high-protein group compared with the intermediate-protein group. Birth weight increased with protein levels up to 69.5 g/day and declined with higher protein intake. A high average prenatal protein consumption resulted in a significant depression of birth weight; in fact, a protein intake of more than 84 g/day on average is more detrimental than a low protein intake. It appears that moderate protein intake is optimal during pregnancy [40].

The effect of soy intake on metabolic status of women with GDM was studied in a randomized clinical trial among 68 women with GDM. Women were randomly assigned to receive either a control diet containing 0.8 g/kg protein (70% animal and 30% plant proteins) ($n=34$) or a soy diet containing the same amount of protein with 35% animal protein, 35% soy protein, and 30% other plant proteins ($n=34$) for 6 weeks. Compared with soy protein consumption, the control group significantly increased fasting plasma glucose ($+1.4 \pm 11.6$ vs -12.7 ± 13.2 mg/dL, $p < 0.001$), serum insulin levels ($+5.0 \pm 11.6$ vs -0.9 ± 10.0 μIU/mL, $p = 0.02$), and HOMA-IR ($+1.2 \pm 2.7$ vs -0.8 ± 2.2, $p = 0.002$) and decreased quantitative insulin sensitivity check index (-0.007 ± 0.02 vs $+0.01 \pm 0.03$, $p=0.004$). Administration of the control diet resulted in a significant difference in serum triglyceride changes ($+31.3 \pm 38.0$ vs $+8.9 \pm 46.1$ mg/dL, $p = 0.03$) compared with soy protein. There were significant

decreases in total antioxidant capacity (-35.0 ± 136.2 vs $+81.8 \pm 188.8$ mmol/L, $p = 0.005$) and glutathione (-41.3 ± 145.7 vs $+53.3 \pm 117.3$ μmol/L, $p = 0.004$) by the control diet intake compared with soy protein. The control diet group had a higher incidence of newborn hyperbilirubinemia (32.4% vs 8.8%, $p = 0.01$) and newborn hospitalization (20.6% vs 2.9%, $p = 0.02$) compared with soy protein. Soy protein consumption in women with GDM significantly improved the glucose homeostasis parameters, triglycerides, and biomarkers of oxidative stress, as well as reductions in the incidence of newborn hyperbilirubinemia and hospitalizations [41].

D Dietary Patterns

1 Low Glycemic Index Diet

In controlling diabetes, it is generally recommended to limit the intake of high glycemic index (GI) foods (highly processed breakfast cereals, instant potatoes, instant noodles, sugar, honey, molasses, corn syrup, candy, sweetened beverages, and fruit, fruit juice, and milk). A diet consisting of low glycemic index (LGI) foods (e.g., whole wheat bread, old-fashioned oatmeal, bran cereal, nuts, legumes, and lentils) may offer a viable alternative to the traditional lower carbohydrate meal plan [42].

An LGI diet in pregnancy was found to have a beneficial effect on neonatal central adiposity and also on postprandial glucose [43]. Grant et al. studied the effect of an LGI diet on blood glucose in women with GDM. Non-Caucasian women were randomized to an LGI ($n = 23$) or control ($n = 24$) diet and followed from 28 weeks gestation until delivery. Glycemic control improved on both diets; however, more postprandial glucose values were within target on LGI (58.4% of $n = 1891$) compared to control (48.7% of $n = 1834$; $p < 0.001$). SMBG postbreakfast was directly related to the prepregnancy BMI at baseline. The authors concluded that an LGI diet was acceptable in this sample and enabled control of postprandial glucose [44].

In another randomized study, 63 women with GDM were assigned to receive either an LGI diet or a conventional high-fiber (and higher GI) diet. Of the 31 women randomly assigned to an LGI diet, 9 (29%) required insulin. Of the women randomly assigned to a higher GI diet, a significantly higher proportion, 19 of 32 (59%), required insulin treatment ($p = 0.023$). However, 9 of these 19 women were able to avoid insulin use by changing to an LGI diet, thus demonstrating that the use of insulin was reduced in almost half [45]. A similar study examined the effect of low glycemic load (LGL) diet with (additional 15 g of wheat fiber) and without fiber in women with GDM requiring insulin. A total of 31 GDM women were randomly assigned to consume either an LGL diet with fiber or LGL diet without fiber. It was found that 7 (38.9%) of 18 women with GDM in the fiber group and 10 (76.9%) in the without fiber group required insulin treatment, thus demonstrating that an LGL diet with added fiber for women with GDM dramatically reduced the need for insulin treatment [46].

The effect of an LGI versus a conventional high-fiber diet on pregnancy maternal metabolic profile in GDM was examined. A total of 99 women (age 26–42 years; mean ± SD prepregnancy BMI 24 ± 5 kg/m^2) diagnosed with GDM at 20–32 weeks' gestation were randomized to follow either an LGI ($n = 50$; target GI ~50) or a high-fiber moderate-GI diet (HF) ($n = 49$; target GI ~60). LGI group achieved a modestly lower GI than the HF group (mean ± SEM 47 ± 1 vs 53 ± 1; $p < 0.001$). At birth, there were no significant differences in birth weight (LGI 3.3 ± 0.1 kg vs HF 3.3 ± 0.1 kg; $p = 0.619$), birth weight centile (LGI 52.5 ± 4.3 vs HF 52.2 ± 4.0; $p = 0.969$), prevalence of macrosomia (LGI 2.1% vs HF 6.7%; $p = 0.157$), insulin treatment (LGI 53% vs HF 65%; $p = 0.251$), or adverse pregnancy outcomes. In this study, an LGI diet and a conventional HF diet produced similar pregnancy outcomes [47].

A randomized study compared the effects of a conventional diet (40% carbohydrate/45% fat/15% protein) to one consisting of a higher-complex carbohydrate (HCC) and low fat (LF) (60/25/15%) Choosing Healthy Options In Carbohydrate Energy (CHOICE) diet in 16 women with GDM. There were no between-diet differences for fasting or mean nocturnal glucose, but 24-hour area under the curve (AUC) was slightly higher (~6%) on the HCC/LF CHOICE diet ($p = 0.02$). The continuous glucose monitoring system (CGMS) revealed modestly higher 1- and 2-hour postprandial glucose on CHOICE (1 hour, 115 ± 2 vs 107 ± 3 mg/dL, $p \leq 0.01$; 2 hour, 106 ± 3 vs 97 ± 3 mg/dL, $p = 0.001$) but well below current targets. After breakfast, 5-hour glucose and insulin AUCs were slightly higher ($p < 0.05$), triglycerides AUC was no different, but the free fatty acids (FFAs) AUC was significantly lower (~19%; $p \leq 0.01$) on the CHOICE diet. This highly controlled study randomizing isocaloric diets and using a CGMS was the first to demonstrate that liberalizing complex carbohydrates and reducing fat still achieved glycemia below current treatment targets and lower postprandial FFAs. The authors concluded that this diet strategy may have important implications for preventing macrosomia [48]. Additional maternal and infant parameters were measured in 12 of the 16 participating women. After approximately 7 weeks, fasting blood glucose ($p = 0.03$) and FFAs ($p = 0.06$) decreased on the CHOICE diet, whereas fasting glucose increased on the conventional diet ($p = 0.03$). Insulin suppression of adipose tissue lipolysis was improved on CHOICE versus conventional diet (56% vs 31%, $p = 0.005$), consistent

with improved insulin resistance. Adipose tissue expression of multiple proinflammatory genes was lower on the CHOICE diet ($p < 0.01$). Infant adiposity was lower with CHOICE versus conventional diet ($10.1 \pm 1.4\%$ vs $12.6 \pm 2\%$) [49].

Overall, a diet higher in complex carbohydrate and fiber and low in simple sugars and fat may be effective in preventing postprandial hyperglycemia, reduce FFAs, improve insulin resistance, and reduce the need for insulin during GDM. In addition, reduced neonatal central adiposity and overall reduced fetal adiposity were observed when using these diets.

2 DASH Diet

In a randomized trial of 52 women with GDM, the study participants were assigned to consume either a control ($n=26$) or a Dietary Approaches to Stop Hypertension (DASH) diet ($n=26$) for 4 weeks. The control diet contained 45−55% carbohydrates, 15−20% protein, and 25−30% total fat. The DASH diet was rich in fruits, vegetables, whole grains, and low-fat dairy products and contained lower amounts of saturated fats, cholesterol, and refined grains with a total of 2400 mg/day sodium. The need for insulin in the DASH diet group was significantly lower than for women in the control group (23% for DASH vs 73% for control group, $p < 0.0001$). Compared to infants in the control diet, those born to mothers following the DASH diet had significantly lower weight (3222.7 vs 3818.8 g, $p < 0.0001$), head circumference (34.2 vs 35.1 cm, $p=0.01$), and ponderal index (2.50 vs 2.87 kg/m^3, $p < 0.0001$) [50]. In a randomized controlled study of women with GDM, the effects of the DASH diet on insulin resistance, serum hs-CRP, and biomarkers of oxidative stress among pregnant women with GDM were studied. The control diet contained 40−55% of its energy as carbohydrates, 10−20% as proteins, and 25−30% as total fats. Consumption of the DASH diet compared with the control diet resulted in decreased FPG (−7.62 vs 3.68 mg/dL; $p=0.02$), serum insulin levels (−2.62 vs 4.32 μIU/mL, $p=0.03$), and HOMA-IR score (−0.8 vs 1.1; $p=0.03$). Increased concentrations of plasma total antioxidant capacity (45.2 vs −159.2 mmol/L; $p < 0.0001$) and total glutathione levels (108.1 vs −150.9 μmol/L; $p < 0.0001$) also were seen in the DASH group compared to the control group [51]. A similar study that compared adherence to the DASH dietary pattern to a control diet in women with GDM resulted in improved glucose tolerance after the glucose load. Decreased A1C levels were also seen in the DASH group compared with the control group. Mean changes for serum total and LDL-cholesterol, and total HDL-cholesterol ratio were significantly different between the two diets. Additionally, consumption of the DASH diet favorably influenced systolic blood pressure. Mean changes of fasting plasma glucose were not significant when comparing the DASH diet with the control diet [52].

Overall, the benefits of the DASH diet in GDM when followed for a 4-week period resulted in improved infant (lower birth weight, head circumference, and ponderal index) and maternal (reduced insulin, hemoglobin A1C, total cholesterol, LDL-cholesterol, oxidative stress, and systolic blood pressure) outcomes.

E Nutrition Management Summary

MNT remains the cornerstone of treatment for GDM and is best prescribed by a registered dietitian or a qualified individual with experience in the management of GDM. Nutrition recommendations for GDM, including gestational weight gain, calorie intake, and macronutrient composition and distribution, are based on limited scientific evidence [53]. Current nutrition practice guidelines for GDM recommend a carbohydrate-controlled meal plan with adequate nutrient content aimed to support maternal needs and fetal growth [54]. In addition to practice guidelines, research findings associating macronutrients and caloric prescriptions to maternal and birth outcomes are also available [29]. Using the current nutrition practice guidelines in combination with new research findings can help the registered dietitian individualize a meal plan that will contribute to the delivery of a healthy infant.

IX PHYSICAL ACTIVITY

Moderate exercise may be an important adjunctive therapy in the management of diabetes in pregnancy, particularly in GDM. The benefits of regular physical activity are found in Table 32.7. ACOG recommends that unless contraindicated, pregnant women should participate in at least 20−30 minutes of moderate-intensity physical activity on most or all days of the week [55]. Physical activity should include aerobic and strength conditioning exercises individualized by the health care provider. A list of exercises deemed safe and unsafe during pregnancy is found in Table 32.8. Women with GDM and taking insulin

TABLE 32.7 Physical Activity Benefits in GDM

- Decreased physical discomforts associated with pregnancy
- Shorter active phase of labor
- Improved sleep
- Decreased stress and anxiety
- Increased insulin sensitivity
- Improved glycemic control
- May help to avoid excessive weight gain
- ↓ physical discomforts

or an oral hypoglycemic agent may need to have carbohydrate-type snacks close at hand should they experience hypoglycemia. To prevent hypoglycemia, exercise should be avoided in the fasting state and during periods of peak insulin activity. Blood glucose levels should be monitored before and after exercise.

TABLE 32.8 Physical Activities Considered Safe and Unsafe During Pregnancy

Safe Activities
• Swimming
• Water aerobics
• Walking
• Jogging
• Low-impact aerobics
• Prenatal yoga
• Stationary bike
• Modified weight training
Unsafe Activities
• Horseback riding
• Contact sports (e.g., soccer, basketball, volleyball)
• Downhill skiing
• Gymnastics
• Bike riding
• Scuba diving
• Sauna, hot tubs, steam room
• Any activity that involves lying on the back

Source: From March of Dimes, http://www.marchofdimes.org/pregnancy/exercise-during-pregnancy.aspx; ACOG Committee Opinion 650, Physical activity and exercise during pregnancy and the postpartum period, 2015.

X PHARMACOLOGICAL THERAPY

Pharmacological therapy is prescribed when MNT, physical activity, and lifestyle intervention in a woman with GDM cannot achieve or maintain target blood glucose levels and/or when the rate of fetal growth is higher than normal [54]. However, there is no conclusive evidence to recommend an optimal time to begin pharmacotherapy. Studies have suggested 1–2 weeks after the initiation of MNT and the glycemic levels remain elevated [54,56].

A Insulin Therapy

For many years, insulin was the only medication approved for use to control blood glucose levels in pregnancy. Although several types of insulin are available today, not all are used in pregnancy. Insulin is either identical to human insulin (regular, neutral protamin Hagedorn [NPH]) or insulin analogs (aspart, lispro, glulisine, determir, glargine, or degludec) and varies in onset, peak times, and duration in the body (see Table 32.9). Until June 2015, all drugs used in pregnancy were categorized by the U.S. Food and Drug Administration using the following lettering system: A, B, C, D, and X. In its place, medications are now based on an individualized risk assessment [57].

The amount of insulin is dependent on the woman's body weight and trimester of pregnancy (Table 32.10). The total amount of insulin is based on the insulin to carbohydrate (ICR) ratio. Rapid-acting analogs are preferred to regular insulin because their action begins 5 to 15 minutes and food is consumed immediately after the injection. The onset and peak times for regular women delays when the

TABLE 32.9 Insulin for Blood Glucose Management

Insulin Type	Onset	Peak Action	Duration
Rapid-Acting			
Lispro	0–15 min	30–90 min	3–6.5 h
Aspart	0–15 min	60–120 min	3–5 h
Glulisine	0–15 min	60–120 min	3–4 h
Short-Acting			
Regular (Humulin, Novolin)	30–60 min	2–3 h	3–6 h
Intermediate-Acting			
NPH	1.5–4 h	4–12 h	12–18 h
Long-Acting			
Glargine	1–2 h	Flat	24 h
Detemir	0.8–2 h	Peakless	Up to 24 h

Source: From E.M. Sisson, D.L. Dixon, Pharmacotherapy for glucose management, in: C. Messing (Ed.), The Art and Science of Diabetes Self-Management Education: A Desk Reference for Healthcare Professionals, third ed., American Association of Diabetes Educators, Chicago, IL, 2014.

TABLE 32.10 Insulin Requirements During Pregnancy

	Insulin Dose (Units/kg Actual Body Weight)
First trimester	0.6–0.8
Second trimester	0.7–1.0
Third trimester	0.8–1.2
With obesity (>150% of desirable body weight)	1.5–2.0, secondary to insulin resistance

Source: From American College of Obstetricians and Gynecologists, Pregestational diabetes mellitus, Practice bulletin No. 60, Obstet. Gynecol. 105 (2005) 680; L. Shields, G.S. Tsay (Eds.), California Diabetes and Pregnancy Program Sweet Success Guidelines for Care. Developed with California Department of Public Health; Maternal Child and Adolescent Health Division, revised edition, chapter updated September 2015.

woman is able to eat. NPH is usually given in 2 doses: one at breakfast and the othe before bedtime. If using a long-acting insulin, it is usually injected once daily during the evening meal or at bedtime. Adjustment is made to the insulin dosage based on the glycemic levels.

B Oral Hypoglycemic Agents

Since the landmark trial on the use of glyburide in the management of GDM, the popularity of oral hypoglycemic agents has increased. This randomized trial reported no difference in the incidence of maternal or fetal complications between glyburide and insulin and no glyburide was detected in the cord serum [58]. Glyburide, a sulfonylurea, releases insulin from the pancreas, thereby lowering blood glucose levels. It is considered a viable alternative to insulin because of its ease of use and the cost benefit. However, later studies have shown that glyburide crosses the placenta and is associated with an increased risk of neonatal intensive care admissions, respiratory distress syndrome, neonatal hypoglycemia, birth injury, and large-for-gestational age infants [59,60]. Glyburide is also associated with hypoglycemia in the mother. The maximum dosage of glyburide in pregnancy is 20 mg. Insulin is initiated if maternal glycemic levels remain above target after the maximum dose has been reached.

Metformin is a biguanide, an insulin sensitizer that acts by decreasing the hepatic production and intestinal absorption of glucose. Although metformin was also shown to cross the placenta, it does not cause maternal hypoglycemia and may be a superior alternative to glyburide [61]. Metformin is associated with mild weight loss and may slightly increase the risk of prematurity [4]. The beginning dose of metformin is 500 mg one or two times daily, with a maximum dose in pregnancy of 2500 mg; there are currently no long-term studies on the effect of oral hypoglycemic agents on the offspring.

XI DIABETES SELF-MANAGEMENT EDUCATION AND BEHAVIORAL APPROACH

Diabetes self-management education (DSME) is a critical element of care for all people with diabetes and is necessary in order to improve patient outcomes. The National Standards for DSME are designed to define quality DSME and to assist diabetes educators in a variety of settings to provide evidence-based education. Because of the dynamic nature of health care and diabetes-related research, these standards are reviewed and revised approximately every 5 years by key organizations and federal agencies within the diabetes education community [62]. The overall objectives of DSME are to support informed decision-making, self-care behaviors, problem-solving, and active collaboration with the health care team and to improve clinical outcomes, health status, and quality of life. One of the guiding principles of DSME is behavioral goal setting as an effective strategy to support self-management behaviors [62]. Diabetes self-management support (DSMS) refers to the support that is required for implementing and sustaining coping skills and behaviors needed to self-manage on an ongoing basis. The initial DSME is typically provided by a health professional, whereas ongoing support can be provided by personnel within a practice and a variety of community-based resources. DSME/S programs are designed to address the patient's health beliefs, cultural needs, current knowledge, physical limitations, emotional concerns, family support, financial status, medical history, health literacy, numeracy, and other factors that influence each person's ability to meet the challenges of self-management [63]. Successful diabetes care requires a systematic approach to supporting patients' behavioral change including (a) healthy lifestyle changes (physical activity, healthy eating, nonuse of tobacco, weight management, effective coping), (b) disease self-management (medication taking and management; self-monitoring of blood glucose and blood pressure when clinically appropriate), and (c) prevention of diabetes complications (self-monitoring of foot health; active participation in screening for eye, foot, and renal complications; immunizations). National DSME standards call for an integrated approach that includes clinical content and skills, behavioral strategies (goal setting, problem-solving), and addressing emotional concerns in each needed curriculum content area [64].

A systematic review of controlled trials evaluated behavior modification interventions to prevent the development of GDM. Nine studies were identified involving such techniques as repetition of information, use of verbal and written educational information, goal setting, and planning, in addition to group and individual counseling

TABLE 32.11 Specific, Measurable, Achievable, Relevant, and Time-Limited Goals in the Management of Diabetes

Type of Goals	Example
Specific goal	I will check my blood glucose
Specific, measurable goal	I will check my blood glucose three times every day
Specific, measurable, achievable goal	I will check my blood glucose before breakfast, after lunch, and before bed time
Specific, measurable, achievable, relevant goal	To avoid hypoglycemic episodes, I will check my blood glucose every day before breakfast, after lunch, and before bed time
Specific, measurable, achievable, relevant, time-limited goal	Starting tomorrow and to avoid hypoglycemia episodes, I will check my blood glucose every day before breakfast, after lunch, and before bed time

sessions. The combination of planning and goal setting was used successfully in improving the diets of women. The findings of the review determined that the use of self-monitoring, goal setting, and achievement appears to be effective in the prevention of excessive gestational weight gain (EGWG), especially when combined with a high frequency of intervention contact, individual attention, and professional involvement [65]. The effectiveness of single versus multiple goal setting for diet and physical activity was evaluated in a randomized controlled trial of overweight or obese adults with T2DM and multiple cardiovascular disease risk factors. At baseline, the multiple-goal group self-selected both diet- and physical activity-related goals, the single-goal group set a single goal, and the control group received information about community health resources. From pre- to postintervention, the single-goal group demonstrated significant improvement in systolic blood pressure and intake of servings of fruits, vegetables, and refined grains (all $p < 0.05$). The multiple-goal group reported significant reduction in percent energy from total, saturated, monounsaturated, and *trans*-fat intake and significant increase in leisure time walking (all $p < 0.05$). The authors concluded that a multiple-goal approach over 4 months can improve dietary and physical activity outcomes, while a single-goal approach may facilitate improvement in one behavioral domain [66].

Patients' perception of collaborative goal setting was evaluated using a semistructured focus group guide. Collaborative goal setting was described by patients as occurring within the context of a caring relationship where patients and health care providers: (1) listen and learn from each other; (2) share ideas; (3) agree on a measurable objective; and (4) support goal achievement. Patients also articulated clear responsibilities for themselves and clinicians and described collaborative goal setting as a process that occurs over time [67]. Therefore, goal setting and goal achievement are instrumental in the management of diabetes and behavior modification. In diabetes management a technique that has been proposed to achieve goals is the use of S.M.A.R.T. (Specific, Measurable, Achievable, Relevant, and Time-limited) goals (Table 32.11).

Specific. The goal should be clearly defined. Stating that people want to have a better glucose control is not specific enough. Focusing on one specific element such as blood glucose control would make the goal specific.

Measurable. Setting a measurable goal would make people accountable for an action that could result in diabetes control.

Achievable. A realistic goal that allows accomplishment.

Relevant. Knowing that a better glucose control could result in positive diabetes outcomes such as A1C within target levels.

Time-limited. A tangible and concrete goal would allow people with diabetes to have a sense of accomplishment.

An example of a S.M.A.R.T. goal is: "I will eat a lunch containing 60 grams of carbohydrates every day during the month" [68].

A Team-Based Approach

According to ADA, persons with diabetes should receive medical care from a team that may include physicians, nurse practitioners, physician's assistants, nurses, registered dietitians, pharmacists, and mental health professionals with expertise in diabetes. It is essential in this collaborative and integrated team approach that individuals with diabetes assume an active role in their care. The management plan should be formulated as a collaborative therapeutic alliance among the patient and family, the physician, and other members of the health care team. A variety of strategies and techniques should be used to provide adequate education and development of problem-solving skills in the various aspects of diabetes management. Implementation of the management plan requires the goals and treatment plan to be individualized and to

take patient preferences into account. The management plan should recognize DSME and ongoing diabetes support as an integral component of care. In developing the plan, consideration should be given to the patient's age, school or work schedule and conditions, physical activity, eating patterns, social situation and cultural factors, and the presence of diabetes and other medical complications [69].

The Chronic Care Model (CCM), an organizational approach to caring for people with chronic disease in a primary care setting, has been shown to be an effective framework for improving the quality of diabetes care [70]. Collaborative, multidisciplinary teams are best equipped to provide care for people with chronic conditions such as diabetes. The key objectives of the CCM are to:

1. Optimize provider and team behavior. The care team should prioritize timely and appropriate intensification of lifestyle and/or pharmacological therapy for patients who have not achieved beneficial levels of glucose, blood pressure, or lipid control.
2. Support patient behavior change. High-quality DSME has been shown to improve patient self-management, satisfaction, and glucose control.
3. Change the care system. Optimal diabetes management requires an organized, systematic approach and involves a coordinated team of dedicated health care professionals.

In addition, a patient-centered communication style should be used that incorporates patient preferences, assesses literacy and numeracy, and addresses cultural barriers to care [71].

A high-performing team is now widely recognized as an essential tool for constructing a more patient-centered, coordinated, and effective health care delivery system. Team-based health care is the provision of health services to individuals, families, and/or their communities by at least two health providers who work collaboratively with patients and their caregivers—to the extent preferred by each patient—to accomplish shared goals within and across settings to achieve coordinated, high-quality care. In conjunction with the patient and family members, health professionals from different disciplines and community providers compose the team. The five personal values that characterize the most effective members of high-functioning teams in health care are: honesty, discipline, creativity, humility, and curiosity. The principles that support high-quality team-based care include shared goals, clear roles, mutual trust, effective communication, and measurable processes and outcomes. These principles are not intended to be in isolation but are interwoven and each is dependent on the others. In addition, these teams require sufficient organizational resources to sustain their work [72]. Developing a clear understanding of and respect for specific roles and responsibilities can be maximized to support achievement of the team's shared goals. The organizational factors that enable establishing and maintaining clear roles include:

- Providing time, space, and support for interprofessional education and training, including explicit opportunities to practice the skills and refine the values that support teamwork.
- Facilitating communication among team members regarding their roles and responsibilities.
- Redesigning care processes and reimbursement to reflect individual and team capacities for the safe and effective provision of patient care needs.

A number of strategies and practices provide the framework for an effective team-based approach. An example of a team-based plan of work is shown in Table 32.12.

A study by Baker et al. found evidence that team training improves patient safety. Three competencies critical for effective team work include:

1. Teamwork-related knowledge: understanding the skills and behaviors needed for an effective team and how they are manifested in a team setting.
2. Teamwork-related skills: the learned capacity to interact with other team members.
3. Teamwork-related attitudes: internal states that influence a team member.

TABLE 32.12 Team-Based Plan to Prevent T2DM After GDM

T2DM Prevention After GDM
Team Composition: Patient, family members, physicians, nurse practitioners, physician's assistants, nurses, registered dietitians, pharmacists, and mental health professionals and support personnel with expertise in diabetes.
Clinical Care: The goal of the multidisciplinary team is to support and treat women with a history of GDM to avoid or delay the onset of T2DM. Women will require an OGTT at 8–12 weeks postpartum.
Team Process: Clinicians collaboratively develop and implement a treatment plan. The team agrees on and implements reliable and timely feedback on successes and failures and on the achievements of the team's goals. These are used to track and improve performance. The team agrees on communication strategies and on a continuous improvement quality plan.

Areas that support effective team-based care are interprofessional education and workforce development, health informatics, and care coordination [73].

B Cultural Aspects

Diabetes management requires individualized, patient-centered, and culturally appropriate strategies. Cultural competency is critical to reducing health disparities and improving access to high-quality health care that is respectful of and responsive to the needs of diverse patients [74]. According to the U.S. Census Bureau, ethnic and minority groups accounted for more than one-third of the population in 2012, with projections indicating that the United States will become a majority-minority nation for the first time in 2044. The minority population is projected to rise to 56% of the total in 2060, compared with 38% in 2014 [75]. Ethnic groups at high risk for developing GDM include Hispanic, African-Americans, Native American, South East Asian, Pacific Islander, or Indigenous Australian groups. Women with GDM who are of Hispanic or African-American backgrounds are more likely to develop hypertension postpartum. A literature review highlights the fact that diabetes management must be individualized and the clinician should be mindful of the impact differences in ethnicity may have on the clinical characteristics and pregnancy outcomes in women affected by GDM, particularly those living in Western countries. MNT plays a key role in the management of GDM and the nutrition prescription should be culturally sensitive. Understanding these differences is critical in the delivery of optimal antenatal care for women from diverse ethnic backgrounds [76].

Cultural competency plays an important role in the communication and counseling carried out by food and nutrition practitioners [77]. To understand the connections between cultural food practices and diabetes among ethnic and racial groups, cultural competence must be gained first. Culture influences values, beliefs, and practices related to food and diabetes. Differences between racial and ethnic groups provide a context for examining cultural food practices and their impact on diabetes management. To best serve the health care needs of racial and ethnic groups with diabetes, health care professionals must acknowledge each group's attitudes, beliefs, and values, including specific knowledge about food habits, preferences, and practices (e.g., holidays, celebrations, and fasting practices). Cultural competence constructs include understanding the language, thoughts, communications, actions, customs, beliefs, values, and institutions of ethnic, racial, religious, or social groups. Recognizing these cultural constructs may better prepare health care professionals to understand their clients' feelings and thoughts about diabetes. By applying the cultural competence constructs, health care professionals may be better prepared to interact with a diverse population requiring diabetes care and education [78].

According to the American Association of Diabetes Educators' position statement on cultural sensitivity, diabetes educators are encouraged to develop a basic understanding of key terminology, such as *cultural sensitivity*, *cultural humility*, *cultural competence*, *multicultural*, *cultural tailoring*, *racial identity*, and *ethnic identity* [79].

Summary recommendations for diabetes education on cultural competency in support of persons affected by diabetes include [80]:

- Acknowledges that cultural perceptions of health can be unique for each individual.
- Considers the context of learning experiences already present when developing collaborative efforts with the patient to identify barriers to diabetes care success.
- Conveys accurate information that is understandable to the learner.
- Proactively addresses limitations to self-management plan adherence and designs/brokers culturally appropriate goals.
- Utilizes educational materials and resources appropriate for culture, age, literacy level, and learning readiness.
- Includes resources that address access limitations to diabetes care needs and considers the milieu in which the care plan is to be executed.
- Incorporates sensitivity and respect when educating all people irrespective of ethnicity, race, age, and socioeconomic status.

XII POSTPARTUM

A Breast-feeding

Research continues to support the positive effects of breast-feeding (BF) on maternal and infant health, as human milk contains the proper balance of nutrients and immunologic agents that closely match infant growth requirements. It is the position of the Academy of Nutrition and Dietetics that exclusive BF provides optimal nutrition and health protection for the first 6 months of life, and BF with complementary foods from 6 months until at least 12 months of age is the ideal feeding pattern for infants. The position recommends BF regardless of the presence of GDM [81].

Recent studies have documented the association of GDM and BF on maternal biomarkers for T2DM and on childhood overweight. The Study of Women, Infant Feeding, and Type 2 Diabetes (SWIFT) investigated whether higher lactation intensity is related to more

favorable blood lipids, lipoproteins, and adipokines (adiponectin, leptin) after a GDM pregnancy independent of obesity, sociodemographics, and insulin resistance. A sample of 1007 women of diverse ethnic backgrounds with previous GDM were divided into exclusive and mostly BF or exclusive or mostly formula feeding (FF) groups, with biomarkers being measured after 6–9 weeks postpartum. Compared to FF groups, BF group reported 5–8% higher HDL cholesterol, 20–28% lower fasting triglycerides, 15–21% lower leptin (all trend p-values <0.01), and 6% lower adiponectin, but only after adjustment for insulin resistance (trend p-value $=0.04$). Findings suggest that lactation has favorable short-term influences on biomarkers for T2DM, except for plasma adiponectin [82]. Furthermore, the long-term effects of lactation and the incidence of T2DM after a GDM pregnancy were evaluated in a subsample of 959 women from the SWIFT study wherein 113 (11.8%) developed incident T2DM. It was found that higher lactation intensity and longer duration were independently associated with lower 2-year incidences of T2DM after GDM pregnancy, thus concluding that lactation may prevent T2DM after GDM delivery [83].

The association of GDM and BF on childhood overweight has been evaluated. One study of 15,710 mother–offspring pairs delivered in 2011 assessed the relationship among maternal obesity, EGWG, GDM, and BF with respect to childhood obesity (BMI >85th percentile) at age 2 years. Logistic regression was used to assess associations between maternal exposures and childhood overweight. Analysis adjusted for exposures and covariates revealed an adjusted odds ratio (95% confidence interval [CI]) associated with childhood overweight at age 2 years of 2.34 (2.09–2.62), 1.50 (1.34–1.68), 1.23 (1.12–1.35), 0.95 (0.83–1.10), and 0.76 (0.69–0.83) for maternal obesity, overweight, EGWG, GDM, and BF $\geq$ 6 months versus <6 months, respectively. The study concluded that GDM and BF $\geq$ 6 months were not associated with childhood overweight at age 2 years. However, maternal prepregnancy obesity or overweight and EGWG were independently associated with an increased risk of childhood overweight and BF $\geq$ 6 months with a decreased risk of childhood overweight at age 2 years [84]. Another study examined the association of GDM and BF on obesity prevalence in predominately Hispanic low-income children (2–4 years). Data from 2295 children (84% Hispanic and 48% female) were obtained from caregivers participating in the Special Supplemental Food Program Women, Infant, and Children. Chi-square and binary logistic regression were used to assess GDM and BF duration with the children's ethnicity, birth weight, age in months, and sex as prior covariates. The results showed that GDM offspring who were breastfed $\geq$ 12 months had a 72% decrease in obesity prevalence (adjusted odds ratio = 0.28, CI 0.89–0.03, $p = 0.05$) [85].

B Prevention of Gestational Diabetes

Evidence on the association between dietary patterns and the risk of GDM has been extensively documented. In the Nurses' Health Study II, the Western diet (characterized by a high intake of red meat, processed meat, refined grain products, sweets, French fries, and pizza) was found to increase the risk for developing GDM. Pregravid intake of red and processed meats was significantly and positively associated with GDM risk, independent of known risk factors for T2DM and GDM. After the adjustment for major risk factors for GDM, those who consumed more than six servings of red meat in a week had more than a 1.7-fold increased risk of GDM compared with those women who consumed less than 1.5 servings of red meat/week (relative risk: 1.74; 95% CI: 1.35, 2.26). In addition, compared to women who consumed one serving per month, those who consumed more than five servings per week of sugar-sweetened cola had a 22% greater GDM risk (relative risk: 1.22; 95% CI: 1.01, 1.47) [86]. Similarly, the consumption of fried foods was associated with the risk of GDM. After adjustment for age, parity, dietary, and nondietary factors, the relative risks (95% CIs) of GDM among women who consumed fried foods 1–3, 4–6, and $\geq$7 times/week, compared with those who consumed them less than once/week, were 1.13 (0.97, 1.32), 1.31 (1.08, 1.59), and 2.18 (1.53, 3.09), respectively ($p <0.001$). The association persisted after further adjustment for BMI ($p=0.01$) [87].

Dietary patterns found to reduce the risk for developing GDM include the prudent diet (characterized by a high intake of fruit, green leafy vegetables, poultry, and fish) [86], Mediterranean patterns (high intake of fruit, vegetables, legumes, fish, and whole grains), and the DASH diet [88–90]. Overall, dietary patterns rich in fruit, vegetables, whole grains, and fish and low in red and processed meat, refined grains, and high-fat dairy were found to be beneficial in the prevention of GDM [91,92].

C Prevention of Type 2 Diabetes Mellitus (T2DM)

Women with a history of GDM are at risk for developing T2DM within 5–10 years after delivery [93]. The ADA—2016 Standards of Medical Care in Diabetes recommends screening women with GDM for persistent diabetes at 6–12 weeks postpartum using the OGTT and clinically appropriate nonpregnancy diagnostic criteria (Table 32.13) [1]. However, less than half of women who had GDM return for diabetes testing [94].

Healthy Babies Need Healthy Moms (HBNHM) is an education program that combines the recommended 2-hour postpartum OGTT with diabetes prevention education for women who had GDM. The 2-hour OGTT is performed during the class and in between venipunctures,

TABLE 32.13 Criteria for Diagnosis of Diabetes Mellitus Using a 75 g OGTT

	Normal Values, mg/dL (mmol/L)	Prediabetes	Diabetes Mellitus, mg/dL (mmol/L)
Fasting plasma glucose	<100 mg/dL	≥100– <126 mg/dL	≥126 mg/dL
	(5.6 mmol/L)	(≥5.6–7.8 mmol/L)	(≥7.0 mmol/L)
75 g OGTT	<140 mg/dL	<140 mg/dL	≥200 mg/dL
	(7.8 mmol/L)	(7.8 mmol/L)	(11.8 mmol/L)

Source: From American Diabetes Association, Standards of Medical Care. Classification and diagnosis of diabetes, Diabetes Care 39 (Suppl. 1) (2016) S13–S22.

the women participate in group education focused on evidence-based diabetes prevention strategies that promote lifestyle behavior, the importance of preconception care for subsequent pregnancies, and the need for follow-up care for women who screen positive for abnormal glucose levels. For women who screen positive for overt diabetes or prediabetes, the educational strategies are directed toward encouraging follow-up care. For women with normal screening results, the program is designed to help prevent the future development of T2DM. Topics covered in the curriculum include pathophysiology of T2DM; diabetes prevention and exercise; BMI, weight loss, and nutrition; preconception counseling and tobacco screening. The nutrition goals of the program are to reduce fat intake to <30% of total energy intake, reduce saturated fat intake to <10%, and increase fiber intake to 15 g/1000 kilocalories [95].

Several healthful dietary patterns, including the alternate Mediterranean Diet (aMED), DASH, and alternate Healthy Eating Index (aHEI), have been inversely associated with T2DM risk and other cardiovascular disease in the general population but rarely investigated among women with a history of GDM (Table 32.14). Validated food-frequency questionnaires were used to measure adherence to a healthful diet of 4413 women from the Nurses' Health Study II cohort with histories of GDM. Results of the study showed that the Mediterranean pattern was associated with 40% lower risk of T2DM (hazard ratio, 0.60 [95% CI, 0.44–0.82; $p = 0.002$]); the DASH pattern, with 46% lower risk (0.54 [0.39–0.73; $p = 0.001$]); and the aHEI pattern, with 57% lower risk (0.43 [0.31–0.59; $p = 0.001$]). Adherence to healthful dietary patterns is associated with lower T2DM risk among women with a history of GDM [96]. In a study by Bao et al., the effect of low-carbohydrate dietary pattern in women with prior GDM with respect to T2DM risk found that a low-carbohydrate dietary pattern, particularly with high protein and fat intake primarily from animal-source foods, was associated with a higher T2DM risk, whereas a low-carbohydrate dietary pattern with high protein and fat intake from plant-based foods was not significantly associated with a risk of T2DM [97].

The Diabetes Prevention Program (DPP) was a major multicenter clinical research study aimed at discovering whether modest weight loss through dietary changes and increased physical activity or treatment with the oral diabetes drug metformin could prevent or delay the onset of T2DM in study participants [98]. The DPP lifestyle intervention has been translated to community settings using the DPP goals of reducing body weight by 7% and engaging in moderate physical activity of 150 minutes per week. The CDC's National Diabetes Prevention Program is an evidence-based behavioral change program, designed to support lifestyle balance, healthy eating, physical activity, and motivational support. The program is delivered by a lifestyle coach, who facilitates the CDC-approved curricula and works to encourage and sustain group interaction so that participants support each other during the year-long program. It includes a handy preparation checklist with materials needed and tasks that are performed to do before participants arrive, as they arrive, and after each session. For each session a lifestyle coach briefly outlines the objectives and key messages. Step-by-step instructions make it easy for lifestyle coaches to facilitate discussions and activities. The curriculum also offers tips on tailoring the sessions to meet participants' needs and preferences, including cultural considerations. The CDC-developed curriculum is available in English and Spanish [99].

As part of the DPP, two studies assessed the effects of metformin on T2DM prevention in women with a history of GDM. In the study by Ratner et al., 2190 women were randomized to either standard lifestyle and placebo, metformin therapy or to an intensive lifestyle intervention. The outcome was the time to development of diabetes ascertained by semiannual fasting plasma glucose and annual OGTT. Results of the study indicated that women with a history of GDM randomized to placebo had a crude incidence rate of diabetes 71% higher than that of women without such histories. Among women reporting a history of GDM, both intensive lifestyle and metformin therapy

TABLE 32.14 Dietary Patterns and Risk for Developing GDM and T2DM After GDM

Stage	Risk	Dietary Pattern	Foods	Relative Risk (95% CI)
Risk for GDM Prepregnancy	High	Western diet	Overall diet: high intake of red meat, processed, refined grain products, sweets, French fries, and pizza	RR: 1.63 (1.20, 2.21); $p = 0.001$ [88]
				RR: 1.74 (1.35, 2.26) [86]
				RR:1.22 (1.01, 1.47) [86]
			Red meat	RR: 1.13 (0.97, 1.32) [87]
			Sugar-sweetened cola	RR: 1.31 (1.08, 1.59)
			Fried foods	RR: 2.18 (1.53, 3.09); p trend <0.001
	Low	Mediterranean diet	Plant-based foods (fruits, vegetables, whole grains, legumes, and nuts), fish and seafood, olive and canola oil and reduced intake of red and processed meats	RR: 0.76 (0.60, 0.95); p trend = 0.004 [90]
		Dietary Approaches to Stop Hypertension (DASH) diet	Plant-based foods (fruits, vegetables, whole grains, legumes, whole grains), low-fat dairy, and reduced intake of sweetened beverages and sodium	RR: 0.66 (0.53, 0.82); p trend = 0.0005 [90]
		Healthy Eating Index diet	Plant-based foods (fruits, vegetables, whole grains, legumes, nuts, whole grains, cereal fiber), higher ratio of white:red meat, PUFA:SFA, multivitamin use, and reduced *trans* fats	RR: 0.54 (0.43, 0.68); p trend = 0.0001 [90]
Risk for GDM During pregnancy	Low	Healthful diet and 150 min/week moderate to intense physical activity	Vegetables, fruits and berries, whole-grain products, rich in fiber, low-fat dairy products, vegetable fats high in unsaturated fatty acids, fish, and low-fat meat products, and a lower intake of sugar-rich foods	RR: 0.64 (0.38, 1.09) [92]
Risk for type 2 DM postpartum	High	Low-carbohydrate diet	Overall diet	HR: 1.36 (1.04, 1.78); p trend = 0.003 [97]
			with high protein and fat intake from animal-source food	HR: 1.40 (1.06, 1.84); p trend = 0.004
			with high protein and fat intake from plant-source food	HR: 1.19 (0.91, 1.55); p trend = 0.50
	Low	Mediterranean diet		RR: 0.60 (0.44, 0.82); $p = 0.002$ [90]
		DASH diet		RR: 0.54 (0.39,0.73); $p = 0.001$
		Healthy Eating Index diet		RR: 0.43 (0.31, 0.59); $p = 0.001$

RR, relative risk; HR, hazard ratio.
Source: From C. Zhang, M.B. Schulze, C.G. Solomon, F.B. Hu, A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus, Diabetologia 49 (2006) 2604–2613.

reduced the incidence of diabetes by approximately 50% compared with the placebo group, whereas this reduction was 49% and 14%, respectively, in parous women without GDM. These data suggest that metformin may be more effective in women with GDM histories as compared with those without GDM [100]. In another similar study, 350 women with a history of GDM and 1416 women with previous live births but no history of GDM were assigned to either placebo, intensive lifestyle intervention (ILS), or metformin. In women with a history of GDM, ILS and

metformin reduced the progression to diabetes compared with placebo by 35% and 40%, respectively. Among women without a history of GDM, ILS reduced the progression to diabetes by 30%, and metformin did not reduce the progression to diabetes. It was concluded that both lifestyle and metformin were highly effective in reducing the progression to diabetes during a 10-year follow-up period. Among women without a history of GDM, lifestyle but not metformin reduced progression to diabetes [101].

XIII CONCLUSION

The diagnosis of GDM should initiate a long-term intervention and diagnostic process to minimize the risk of developing diabetes later in life. Pregnancy complicated by diabetes represents a window of opportunity for the implementation of nutrition education and a physical activity program to modify lifestyle patterns toward healthier habits for the woman and her family. Furthermore, the prevention of T2DM and subsequent gestational diabetes could begin with a healthy lifestyle in the interconception period and last into the future.

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Chapter 33

Interaction of Genetic Factors With Nutrition in Cancer

Jo L. Freudenheim and Emily Gower

University at Buffalo, The State University of New York, Buffalo, NY, United States

I INTRODUCTION

Each year, there are more than 14.1 million new cases of cancer and approximately 8.2 million deaths from cancer worldwide. In the United States alone, there are approximately 1.6 million new cases annually [1]; there are approximately 15.5 million people alive in the United States with a cancer history. Approximately 67% of those cancer patients survive for 5 years or longer after diagnosis [2]. Therapies to cure cancer continue to improve, but prevention is clearly an essential strategy in ameliorating cancer-related morbidity and mortality.

There are considerable differences in the rate of different kinds of cancers worldwide. Given those differences and changes in rates of cancer when populations migrate from one environment to another, it is generally agreed that most common cancers are caused, in large part, by external factors, including diet, that have the potential to be altered [3,4]. Genetic factors alone do not explain the observed variation in rates. An understanding of how dietary factors and overall nutrition contribute to cancer is one part of understanding cancer prevention and prognosis.

There is accumulating evidence that carcinogenesis is a complicated process involving interactions of an individual's endogenous milieu, including his or her genetic makeup, with other exogenous exposures including diet and nutrition. One simplified model for an interaction of diet and genetics is shown in Fig. 33.1. In this case, there is a different rate of increase in risk with a dietary exposure for those with a particular gene that increases the likelihood of cancer compared to those without the gene. Both factors affect the risk of the disease [5].

In conceptualizing what causes cancer, it is crucial to understand that a cancer may have more than one cause. For example, both occupational exposures and diet could contribute to the etiology of one kind of cancer and even to the cancer of one individual, and either a change in the occupational exposure or a change in diet might prevent that cancer or reduce the risk. Similarly, both dietary and genetic factors may contribute to the same cancer. Sometimes those contributions are additive. That is, the risk for both is the sum of the risks for each. In other cases, the contributions are interactive. That is, the genetic factors interact with the nutritional factors so that the risk is not the same as the sum of each.

It is estimated that nutritional factors contribute to between 30% and 40% of tumors [3]. Genetic factors likely play a role in many, if not all, cancers. Even when an exposure is clearly required for a particular kind of cancer, genetic factors often play a role in determining who, among exposed individuals, develops that cancer. In examining the interactions of nutrition and cancer, it is also important to recognize that cancer is a collection of diseases that differ in their causes; even among cancers at one site such as breast cancer, there are several different kinds of breast cancer with differences in risk factors and underlying etiology.

II BACKGROUND AND DEFINITIONS

Cancer is a disease of uncontrolled growth of cells with spread to other tissues [6]; it is characterized by a number of changes in cells. Tumor cells have their own growth signals, do not respond to signals inhibiting growth, can replicate indefinitely (replicative immortality), can avoid programmed cell death (apoptosis), can alter energy metabolism, can avoid immune surveillance, can invade

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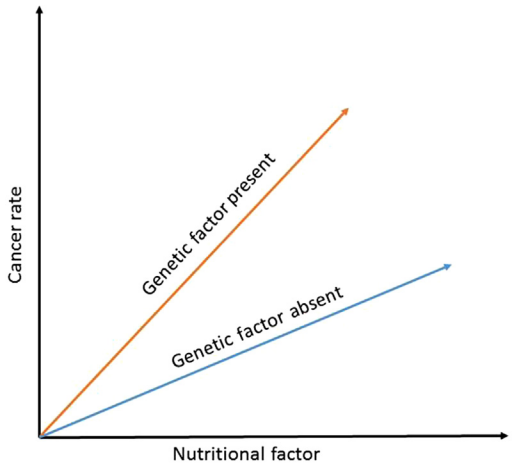

FIGURE 33.1 The effect of a dietary factor or other nutritional exposure on the rate of cancer might vary depending on the presence of a particular genetic factor. *Adapted from V. Andersen, R. Holst, U. Vogel, Systematic review: diet-gene interactions and the risk of colorectal cancer, Aliment. Pharmacol. Ther. 37 (4) (2013) 383–391.*

other tissues (metastasis), and can develop a blood supply for those metastases (angiogenesis). Inflammation and instability in the genome leading to mutations and other alterations are also part to the observed changes [7]. In the process of carcinogenesis, there is an accumulation of genetic and other alterations that lead to these significant changes in the functioning of the cell.

Genes are the basic unit of heredity; each gene has its own location in the DNA on a particular chromosome [8]. A *mutation* is a structural change in the base pair sequence of DNA, the chromosomal material that provides the code for gene expression [8]. In tumor cells, there are usually a number of mutations of the DNA. In addition, there may also be *epigenetic* changes. These are changes other than changes to the DNA base pair sequences. Like mutations, epigenetic changes can also affect gene structure, function, and expression. One such change is *altered DNA methylation*, the addition or loss of methyl groups on the DNA, which can affect gene expression. *Hypermethylation* of the promoter region of tumor suppressor genes can lead to silencing of the gene. This change is common in tumors and may be as important a mechanism as mutation in carcinogenesis. Other epigenetic changes include methylation or acetylation of *histones*, proteins associated with DNA that affect its structure [8]. There may also be alterations in copy number—that is, the number of copies of the same gene—altering the overall expression. In cancer cells, there may also be the loss of one or more sections of the genome as well as translocation of sections of the genome from one location to another.

Genotype refers to an individual's genetic structure, based on his or her DNA [8]; it is the nucleic acid sequence of the DNA. Generally, each individual has two copies of each gene, one inherited from each parent. In some but not all cases, differences in genotype provide information regarding protein activity. For example, genotype for a faster or slower version of a particular enzyme would influence the rate of the reaction catalyzed by that enzyme. Other factors besides genotype can also influence activity of an enzyme or other protein. These include the rate of transcription of the gene, available concentration of substrate(s), and the rates of synthesis and degradation of the protein. *Phenotype* refers to observable characteristics; it represents the expression of the genotype, including the effects of interactions with other genes and with the environment [8]. Examples of phenotype are general characteristics such as height and body weight, as well as other, more specific characteristics such as the activity of an enzyme or receptor, or blood vitamin concentration.

Agreement between genotype and phenotype often is not exact, as described in more detail later. Phenotype may not be dependent on a single gene but, rather, the result of genotype of a number of different genes in a pathway, combined with the impact of external exposures. For example, height is influenced by a number of genes and by external factors including nutrition.

When there is an *interaction*, the effect of a factor differs depending on the presence or absence of another factor. In many instances, there are gene–gene interactions as well as gene–environment interactions. For example, a person may have the genotype for lower activity for a particular enzyme. However, there can be other exposures including dietary factors that induce greater expression of that enzyme. Phenotype, the activity of the enzyme, would depend on the combination of the genotype (faster or slower) and the other exposures (e.g., higher intake of a dietary exposure that increases the amount of the enzyme that is present). The measurement of phenotype is of interest because it gives an indication of the true level of exposure. Thus, for example, measurement of serum vitamin D would be a phenotype of interest, which might be more informative than measurement of genotype for the number of genes related to vitamin D status. In other cases, genotype may be the preferable measure. Genotype may give a better indication of lifetime exposure, whereas phenotype (gene expression) may be influenced by recent exposures. In particular, when study participants have a disease, phenotype may be less informative if the disease process influences it.

Penetrance of a genetic factor refers to the likelihood that those with a specific genotype will exhibit a particular phenotype in given environmental circumstances [8]. A gene with very high penetrance is one for which virtually everyone with the gene has the expressed trait, whereas a gene with low penetrance is one for which there is a lower likelihood of the trait being expressed.

Some individuals are at greater risk for certain types of cancers. This increased risk can be related to differences in exposure to cancer-inducing or -protecting agents, to the individual's genetic makeup, or to a combination of these factors. Genetic factors vary widely with regard to the magnitude of their impact. Some inherited mutations greatly increase the risk of cancer; others cause smaller increases in risk. Some mutations may only increase risk in the presence of a particular exposure.

Examples of inherited mutations with high penetrance are mutations identified in the *BRCA1* and *BRCA2* genes. Particular mutations in these genes have been shown to be strongly related to risk of breast and ovarian cancer. It is estimated that carriers of the *BRCA1* and *BRCA2* genes have a 45−65% risk of breast cancer by age 70 years; that is, many, but not all, women with this mutation will develop breast cancer. Relatively few individuals in the population carry this factor (less than 1%). Therefore, although those with the gene are at high risk, only approximately 5−10% of women with breast cancer have this mutation [9].

There is a considerable range in the impact of genetic differences on phenotype. Other genetic variants that are more common and that have weaker effects on risk are referred to as *genetic polymorphisms*. A gene that is polymorphic has more than one form; its structure varies among individuals in a population. Many polymorphisms are silent, not affecting phenotype. Other polymorphisms can alter the activity of a protein and/or may affect its interaction with other compounds including nutrients. The change in one nucleotide is referred to as a *single nucleotide polymorphism* (SNP). There are frequently one or more polymorphisms found in a gene. The frequency of the occurrence of a genetic polymorphism among individuals within a population may vary greatly depending on the ancestry of the individuals in that population. Generally, polymorphisms have less impact on cancer risk than do highly penetrant mutations such as *BRCA1/2*. However, when they are common, they may still have an overall significant effect on the rate of disease in a population. Common polymorphisms may affect the response to an exposure so that their effect on risk would be evident only when that exposure or exposures are present, referred to as a gene−environment interaction. When there are gene−environment interactions, an understanding of both the genetic factor and the environmental exposure is needed in order to understand disease etiology and prevention.

Studies examining associations with disease risk of a single gene or of several genes in a pathway are called *candidate gene* studies. In these studies, genes are selected based on a hypothesis regarding a likely mechanism. In *genome-wide association studies* (*GWAS*), instead of examining SNP-outcome associations in particular genes, genetic variation across all of the chromosomes is examined. For this "agnostic" approach, hundreds of thousands of SNPs are examined in one study to understand which, if any, are associated with a particular outcome, such as a disease (e.g., cancer) or a biological marker (e.g., concentration of a vitamin in blood or body mass index). To be effective, such studies must include genetic data and outcome data from hundreds of thousands of participants [10]. When a particular SNP is identified from a GWAS, it does not necessarily mean that the identified SNP increases the likelihood of the outcome. SNPs can be in *linkage disequilibrium*, which means that there are SNPs that are often found together; the SNPs are not randomly distributed across the population. If there is linkage disequilibrium, an identified SNP, with a strong association with the outcome, may be an indicator for another SNP that is causally related to the outcome (Fig. 33.2).

- Examination of hundreds of thousands of genetic variants at one time
- Examination also possible of variation in epigenetics (e.g., DNA methylation)
- Compare frequency of each variant for those with and without an outcome of interest
- Outcome can be a disease (e.g., bladder cancer cases vs controls without bladder cancer)
- Outcome can be a nutrition-related variable (e.g., blood vitamin D binding protein high vs low, intake of macronutrients)
- Variants with strongest statistical associations may be investigated further to better understand findings including identifying variant more specifically and understanding mechanisms of action

FIGURE 33.2 Genome-wide association studies.

Similar to the methods for GWAS of genetic variation, genome-wide variation in DNA methylation can be studied. In a genome-wide study of diet in relation to DNA methylation, generally the dietary factor would be considered the exposure and the genome-wide methylation the outcome. For example, there are studies examining the association of obesity or vitamin intake with DNA methylation, examining hundreds of thousands of locations in the genome to identify those that are associated with the nutritional exposure.

Several terms have been developed to describe the interaction of food components with genes. *Nutrigenomics* is the broadest term, describing the interaction of dietary components with genes. Included in this term is *nutrigenetics*, the study of the impact of genetic variation on response to dietary components; *nutritional epigenetics*, the study of interactions of food-derived compounds with epigenetic alterations—for example,

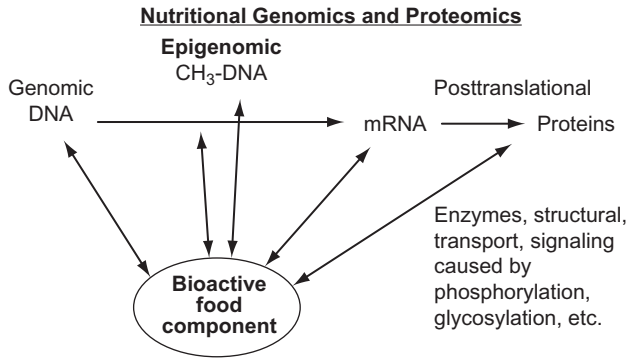

FIGURE 33.3 Bioactive food components may interact with genes and their products in a number of different ways, including interactions with the coding DNA, with other parts of the chromosome structure, with the messenger RNA, and with the protein product after translation. *From J.A. Milner, Molecular targets for bioactive food components, J. Nutr. 134 (2004) 2492S–2498S. Copyright The American Society of Nutrition. Reprinted by permission.*

Genes:

- can affect biological effect of a nutrient or other bioactive food component
- can interact with each other
- can interact with other environmental exposures

Nutrients and other bioactive food components:

- can affect gene expression
- can interact with each other
- can interact with other environmental exposures

FIGURE 33.4 Diet and gene interactions.

effects of nutrition on DNA methylation and on chromatin structure; and *nutritional transcriptomics*, the study of the impact of dietary components on gene transcription. In addition, dietary components may affect protein structure posttranslation; the study of these interactions is *nutritional proteomics* (Fig. 33.3) [11,12].

III MECHANISMS OF DIET–GENE INTERACTIONS

A Overview of Interactions

In the exploration of the role of dietary factors in carcinogenesis, both essential nutrients and other compounds in foods with biological activity may be important. Interactions of nutritional and food-derived bioactive compounds with genetic factors may operate in two directions. Genes can affect the action of compounds of dietary origin (e.g., increasing or decreasing the amount needed for good nutritional status), and the dietary compounds can affect the action of genes (e.g., leading to increased or decreased expression of a particular gene) (Fig. 33.4).

To understand these processes, several layers of complexity need to be taken into account. Individual bioactive food components may have more than one action, in some cases interacting with other food components and with more than one gene. Food composition can differ significantly, depending on growing conditions, genetic differences of the foodstuff, storage and cooking, and other food processing [13]. After consumption, there can be interactions of a particular bioactive compound with other food constituents, with other environmental exposures (e.g., from air and water), and with genes. Furthermore, genes can interact with each other. The resulting metabolic environment derives from these numerous interactions of factors of both endogenous and exogenous origin. In understanding the role of a particular food component, examination of its interactions with other food components and with one or more metabolic pathways may be necessary, as well as possible consideration of differing actions depending on dose or on tissue site.

This complexity is only partially understood for most of the bioactive compounds that have been studied. Single food components and single foods have been studied in relation to single genes for cancer. A limited number of studies have examined the impact of a group of foods or of dietary patterns on risk or prognosis. There have been a limited number of GWAS, examining thousands of SNPs in relation to nutritionally significant outcomes, for example, with the blood concentration of a particular nutrient. These studies are essential first steps; there is much to be learned as the more complex systems of interactions are examined. Presented here is information about the impact of genetic variation on the availability and utilization of dietary compounds in relation to carcinogenesis and about the impact of diet on genetic factors in relation to carcinogenesis [14].

B Genetic Variation in Relation to Metabolism of Food Components

1 Carcinogen Metabolism

Genes may impact the metabolism of carcinogens including both compounds occurring naturally in foods and others that contaminate foods. If an individual has genetic variants that result in slower metabolism of a carcinogen, that individual will be more affected by a given dose of that carcinogen than would someone who can metabolize and excrete the carcinogen more rapidly. Conversely, if an individual has genetic variants that result in more rapid metabolism of a carcinogen, then he or she may be less affected by the same dose than someone who does not have that variant.

Enzymes related to carcinogen metabolism and excretion are divided into two groups: phase I and phase II.

Phase I enzymes activate the compound, and phase II enzymes attach polar groups to the activated compound so that it will be more water soluble and can be excreted in the urine. Many of the phase I enzymes are in the cytochrome P-450 (CYP) family. Phase II enzymes include glutathione-*S*-transferases (GSTs) and *N*-acetyltransferases (NATs). In some cases, phase I activation leads to the production of compounds with greater carcinogenic potential. Often, foods contain procarcinogens, compounds that are carcinogenic after metabolic activation. The process of metabolic activation may vary depending on genetic factors. For example, heterocyclic amines are a group of compounds found in meat cooked at high temperatures, primarily "well-done" meat and fish. There is some, although not consistent, evidence that consumption of heterocyclic amines may increase the risk of cancer. For example, there is evidence of an association between intake of these compounds from meat with increased risk of colorectal [15] and of prostate cancer [16]. This association may depend on genetic factors. Metabolism and excretion of heterocyclic amines involve CYP1A2, *N*-acetyltransferases 1 and 2 (NAT-1 and -2), and UDP-glucuronosyltransferases [17]. There is evidence that the association of colorectal cancer with intake of heterocyclic amines differs depending on genetic variation in NAT-1 [18]. In another study, there was an interaction of NAT-1 genotype and intake of dietary mutagens in relation to prostate cancer risk [19]. The relationships between these phase I and phase II enzyme systems are shown in Fig. 33.5 [20,21].

Other kinds of genetic variation may also affect association of nutrition and diet with cancer outcomes. Copy number variation—the number of copies an individual has of a particular gene—may also affect metabolism. For example, there is evidence that the number of copies of the amylase gene is correlated with the levels of the amylase protein in saliva [22]. Dietary intake of nutrients important in one-carbon metabolism may impact DNA methylation. One study showed an association of folate and green vegetable intake with promoter methylation in sputum from apparently healthy smokers [23]. In another, there was no association of folate intake with DNA methylation of three particular genes in breast tumors [24]. Genome-wide studies of DNA methylation may provide more insight as to changes associated with diet. For example, in a study of elderly individuals, there was evidence that supplementation with folic acid and vitamin B12 affected methylation of particular genes and regions of chromosomes [25].

2 Metabolism of Nutrients and Other Food Components

There can be significant genetic variation among individuals in the absorption, tolerance, transport, and excretion of nutrients and other bioactive food components as well as their metabolism [13,26]. All of these can alter effects of intake of the same amount of a food component by individuals with different genes. Furthermore, genetic factors may alter food preferences and therefore food consumption [27]. In one study of more than 37,000 individuals, SNPs were identified that were associated with differences in intake of macronutrients providing evidence that macronutrient intake may be affected by genetic factors [28].

The impact of a particular genetic polymorphism may differ for different compounds. It is even possible for a genetic variant to protect against cancer for exposure to one compound and increase risk for another. For example, the GSTs are phase II enzymes that catalyze transformation of carcinogens such as polycyclic aromatic hydrocarbons (PAHs) for excretion. For those with higher exposure to PAHs, including PAHs of dietary origin produced in the cooking of foods, more rapid metabolism would be advantageous. Some of the GSTs also deactivate other phytochemicals that are thought to exert protective effects. Included in the latter group are the isothiocyanates from cruciferous vegetables, which are metabolized by GST, also resulting in their excretion [27]. For those compounds, GST metabolism resulting in slower excretion would be advantageous.

Genetic variation in the metabolic pathway for a nutrient or bioactive food component can affect the physiologic impact of intake of that compound. For example, if a vitamin plays a role in cancer prevention, genetic differences in the receptor for the vitamin could also affect the susceptibility of an individual to cancer. There are commonly occurring genetic differences in the vitamin D receptor that may have functional significance. A number of studies have examined the association of those variants with risk of several cancers. There is some evidence that genetic variation in the receptor is associated with cancer risk [29]. Genetic factors may influence status of a nutrient. A GWAS was done to identify the SNPs most associated with the blood concentration of the vitamin D binding protein. More than 590,000 SNPs were examined for approximately 1300 study participants. Some of the results of that study are shown in the plot in Fig. 33.6. From that plot of the statistical association of the D binding protein with each of the SNPs, it is possible to identify the ones most strongly associated with blood concentration of the binding protein [30]. In another GWAS examining a large number of SNPs in association with vitamin D in blood, several genetic variants were found to be associated with circulating 25-hydroxyvitamin D [31].

There has been considerable exploration of genetic variants in the one-carbon metabolism pathway. Genetic variation in this pathway affects blood folate concentrations and folate transport and utilization. This pathway

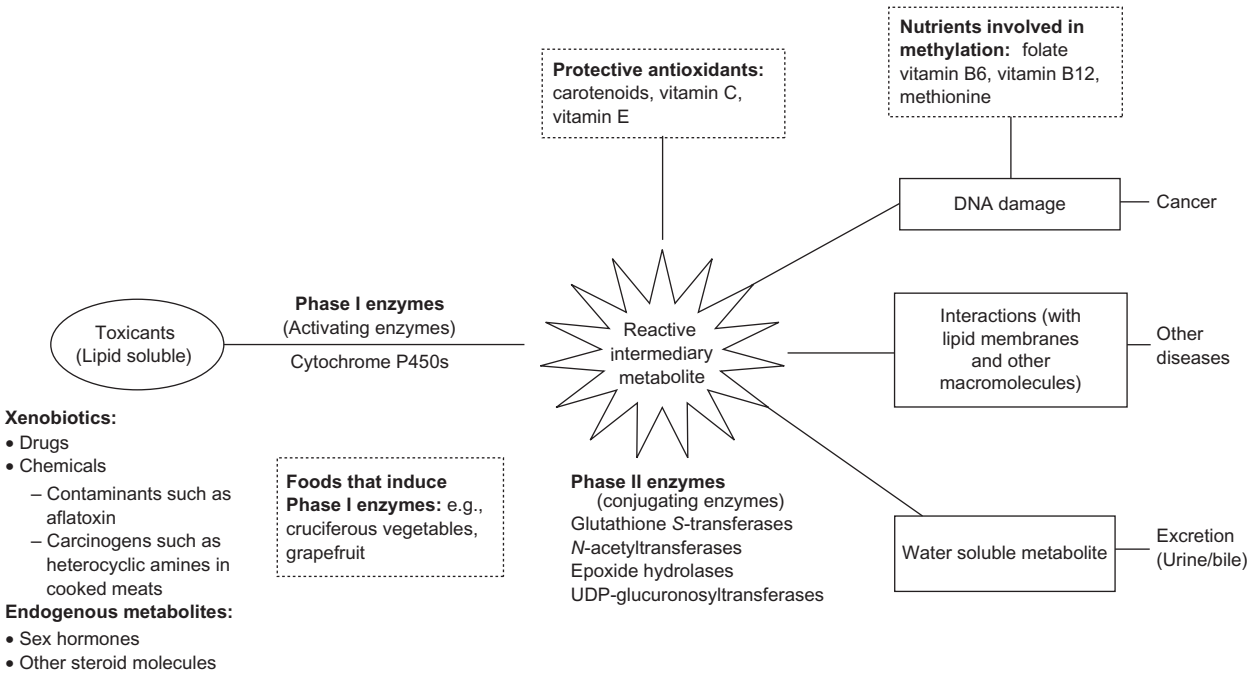

FIGURE 33.5 Interrelationship between the biotransformation enzyme systems. *From R.E. Patterson, D.L. Eaton, J.D. Potter, The genetic revolution: change and challenge for the dietetics profession, J. Am. Diet. Assoc. 99 (1999) 1412–1420. Copyright The American Dietetic Association. Reprinted by permission.*

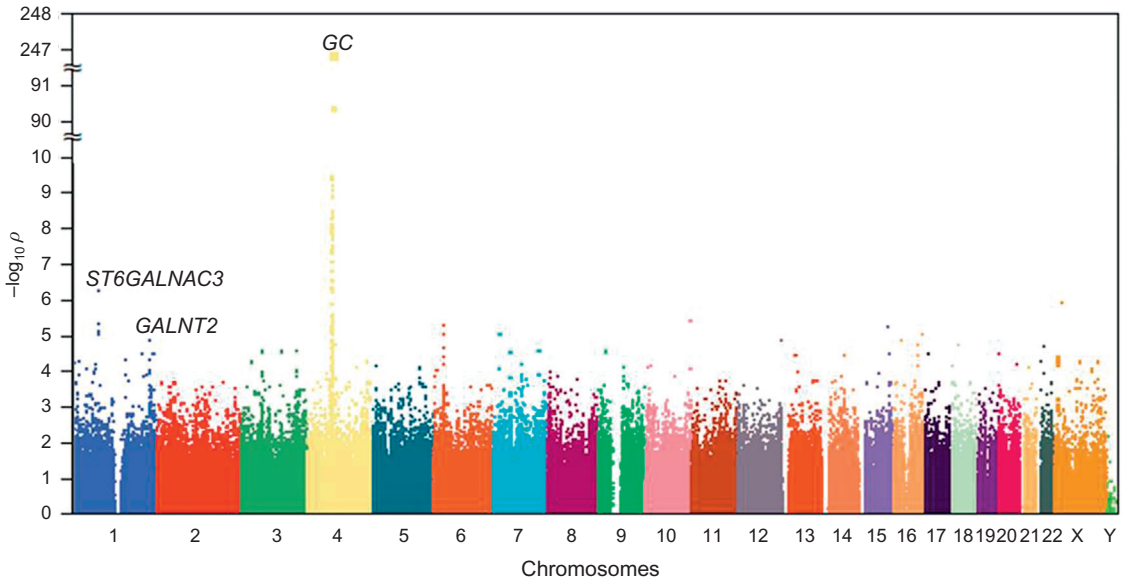

FIGURE 33.6 GWAS of SNP with blood concentrations of the vitamin D binding protein. For each SNP location on the chromosome (*x*-axis) there is a *p*-value for the association (*y*-axis). There were two SNPs, both in the binding protein (chromosome 4) that were strongly associated with the blood concentration of the binding protein. *From K.A. Moy, A.M. Mondul, H. Zhang, S.J. Weinstein, W. Wheeler, C.C. Chung, S. Mannisto, K. Yu, S.J. Chanock, D. ALbanes, Genome-wide association study of circulating vitamin D-binding protein, Am. J. Clin. Nutr. 99 (2014) 1424–1431. Reprinted by permission.*

also includes the synthesis of nucleic acids. Genetic variation may therefore also affect the synthesis of purines and pyrimidines for DNA synthesis as well as potentially impacting gene methylation and the expression of genes. There is some, although not consistent, evidence of an association of variants in genes in this pathway and risk of several cancers [11]. However, in one study examining genetic variation of genes involved in one-carbon

metabolism, there was no association with the prevalence of DNA methylation in breast tumors [32].

Another example of a gene that affects nutrient utilization is the *HFE* gene. This gene contains a variant, common among Europeans, that affects iron storage and contributes to the etiology of hemochromatosis [33]. There is also evidence of genetic variants that are associated with total iron binding capacity and percent iron saturation [34]. Iron overload is associated with increased risk of hepatocellular carcinoma [35].

There is genetic variation in several of the enzymes involved in alcohol metabolism. A variant in the gene for aldehyde dehydrogenase (ALDH2) is carried by approximately 50% of Asians and significantly impacts alcohol metabolism. Among those with the variant, there is greatly reduced clearance of acetaldehyde; those who are either heterozygous or homozygous for the variant experience an adverse reaction, an intense flushing, when they consume alcohol. The variant has been shown to be associated with both lower alcohol consumption and alcoholism [36]. Acetaldehyde is a carcinogen. There is evidence of an interaction between *ALDH* genotype, alcohol intake, and risk of cancer at several sites [37].

Another example of an interaction of diet, genetics, and a health outcome related to cancer is that of sugar-sweetened beverages and obesity. There are number of genetic variants that have been found to be associated with obesity; obesity increases the risk of a number of kinds of cancer. In one prospective study, investigators looked at a signature of those genes associated with obesity. They found that the association of the gene signature with obesity was stronger among those who consumed more sugar-sweetened beverages; there was a statistically significant interaction of the selected genetic variants with the sweet beverages and likelihood of obesity [38]. It appears likely that we are just beginning to understand how genetic variation affects nutrient metabolism and affects the role of nutrients in pathways relevant to carcinogenesis. Better understanding of those interactions may help in providing clearer guidance regarding appropriate prevention strategies. It may also be that there will eventually be personalized prevention programs, identifying individuals with particular genetic signatures that indicate that they have different requirements for nutrients that impact cancer prevention.

C Interactions of Bioactive Food Components With Gene Functions in Relation to Carcinogenesis

1 Mutations and Metastasis

Nutrients and other bioactive food components appear to contribute both in positive and negative ways at every stage of the carcinogenic process, including interactions with DNA mutations and tumor metastasis. Some DNA mutations result from carcinogens that bind to the DNA, forming DNA adducts. Carcinogens in foods, such as the PAHs described previously, are one potential source of these carcinogens. Binding to the DNA, such adducts may interfere with DNA replication and result in mutations or deletions in DNA structure. Dietary factors may also affect the formation of adducts, such as those from smoking. For example, there is evidence that among smokers, adduct formation is more likely among those with lower β-carotene intake. Furthermore, genetic variation may also affect this association. The association between adduct formation and β-carotene intake appears to be limited to individuals who carry homozygous deletions for the gene for glutathione-*S*-transferase (*GSTM1null*) [39].

Oxidative stress may also affect carcinogenesis. There is evidence that reactive oxygen species from both exogenous and endogenous sources can cause DNA mutations. Diet can affect exposure to reactive oxygen species both positively and negatively. With greater energy consumption, there is a tendency for a greater number of oxidized DNA bases [3]. Antioxidant vitamins and certain other enzymes are part of the body's defense against these oxidative processes. As described above, the concentration of those vitamins may be affected by genetic factors. The enzymes too may have more or less reactivity depending on genetic factors. Those who have genetically weaker defense systems against oxidation may benefit more from increased intake of antioxidant vitamins, such as vitamins C and E. For example, superoxide dismutase is important in the control of endogenously produced reactive oxygen species; manganese superoxide dismutase (MnSOD) is found in the mitochondria, a location for production of oxidative species. The gene coding for MnSOD is polymorphic, and a study suggests that one variant of this gene may be associated with increased risk of premenopausal breast cancer but primarily among women with a low intake of antioxidant vitamins from fruits and vegetables [40].

Dietary factors may also influence genes that are related to metastasis. Catechins—found particularly in green tea but also found in black tea, apples, and chocolate—can downregulate enzymes important in the development of metastases [41]. Other vitamins including vitamin A impact gene expression including expression that plays a role in pathways related to carcinogenesis such as cell differentiation and control of cell death [42]. Vitamins with antioxidant properties including vitamins C and E and green tea extract may affect genes significant in carcinogenesis including those not directly related to control of oxidation [43].

Genetic variation may also affect genetic stability and DNA repair. There is evidence that nutrients may be able to promote stability and thereby prevent carcinogenesis.

Although the data regarding this potential mechanism are rather limited, there is evidence of an effect of diet on functions essential to the maintenance of DNA integrity [44].

2 Gene Expression

In addition to effects on gene structure and mutations, nutrients and other dietary factors may also impact cancer by altering gene expression. For example, the *CYP1A2* gene can be induced by indole-3-carbinol found in cruciferous vegetables, by heterocyclic amines in cooked meats, and by PAHs found in grilled meat. *CYP1A2* is also inhibited by the compound naringenin, which is found in grapefruit [45]. Therefore, metabolism of heterocyclic amines by the enzyme encoded by the *CYP1A2* gene may depend not only on the genetic variant of *CYP1A2* that is carried by an individual but also on the individual's intake of these other food components that affect *CYP1A2* expression. Similarly, consumption of a diet that is low in fat and low in glycemic load was shown to be associated with alterations in gene expression in the prostate epithelium in humans [46].

Interactions with gene expression may be part of the normal functioning of the food component and of the gene, leading to either upregulation or downregulation of the gene expression. Vitamins A and D interact with the promoter regions of a number of genes, influencing their expression and thereby affecting a host of cellular functions, including cell proliferation and differentiation and DNA replication and repair [47]. A group of receptors called peroxisome proliferation-activated receptors (PPARs) affect the expression of genes important in fatty acid metabolism and tumorigenesis. One of these, PPARα, is activated by oxidized fats, affecting expression of genes important in fatty acid synthesis [48]. The intracellular energy balance can also affect gene expression [49]. A number of other nutrients and other food components have been shown to affect gene expression in cell culture, including vitamin E, biotin, zinc, flavones, and catechins [49].

3 Epigenetic Alterations

Alterations of DNA structure other than base pair changes can also affect gene expression. Important among these are DNA methylation and alterations in histones. There is evidence of possible contributions to carcinogenesis by an overall decrease in DNA methylation (global hypomethylation). In addition, an increase in the methylation (hypermethylation) of the promoters of certain genes, particularly tumor suppressor genes, may contribute to the development of cancer by silencing them. Dietary factors involved in one-carbon metabolism may affect this methylation. These factors include folate, vitamins B6 and B12, biotin, choline, and methionine as well as alcohol. Alcohol can adversely affect folate status and indirectly affect methyl availability. In one study, there was more methylation of DNA in breast tumors from women who drank more than in the breast tumors from women who drank less [50]. In addition to nutrients related to methyl availability, there is also evidence that genistein, coumestrol, and a high-fiber diet may all affect methylation patterns [51]. Mechanisms for both hypo- and hypermethylation are not well understood and may involve other factors of both exogenous and endogenous origin.

In addition to methylation changes, dietary factors may impact other epigenetic factors including effects on the histones. Inhibitors of histone deacetylators include butyrate (formed by the bacterial fermentation of fiber in the colon), diallyl disulfide (from plants in the allium family, including garlic), and sulforaphane from cruciferous vegetables [11]. Resveratrol, found in grapes and grape products, may affect carbonylation of histones. Diet may also affect gene expression through other epigenetic mechanisms. One such mechanism is the impact of diet on microRNA expression, affecting expression of a number of other genes including tumor suppressor genes and oncogenes [52].

The fields of genetics in general and nutrigenetics in particular are changing rapidly; numerous discoveries are leading to major shifts in our understanding of the role of genetic factors in cancer risk and of the interaction of dietary and other exposures with those genetic factors. There is evidence that a number of pathways involving diet and gene interactions may ultimately impact disease. Other pathways of interaction will likely be identified, and some of those detailed here may be determined to be of lesser importance to humans. Because much of our understanding of gene−environment interactions in relation to cancer is based on relatively new information, it is important to recognize that much of the data will be subject to reinterpretation and that an understanding of the methodological issues involved is key.

IV METHODOLOGICAL ISSUES

Data regarding interactions of diet and genes and cancer come from a number of sources, including studies of normal and tumor cells in culture, animal models, and human epidemiologic studies. Each of these study modalities has advantages and disadvantages. For cell culture studies, the concentration of an agent may be very important, and the studied concentration may or may not relate to physiological conditions in the tissue of interest. Also important to consider are the duration of the dosage and the type of cells used. Some cultured cells may also have characteristics different from what occurs in vivo. In addition, results may vary depending on the cell model used, even for different models of the same cancer site.

Animal models are an important source of understanding of the underlying mechanisms of carcinogenesis. Again, however, in interpretation of findings, considerations of the dose and duration of administration of an agent are important. Also important is an understanding of differences between the physiology of a particular animal used in the experimental systems and humans. In addition, the complex mix of exposures humans experience over an extended period of time may be of importance and may not be replicated in laboratory animal or cell culture studies. Even when the animal model can provide very good information, there may be important caveats that inform the conclusions that can be made from the experiments. For example, there are numerous studies of the administration of high-fat diets to animals leading to mammary tumor development. One limitation of such studies that is often not noted is that the observed increases in mammary tumors were found only when the high-fat diets were administered to virgin animals. In parous animals, the high-fat diets did not increase mammary tumors [53]. Such caveats are important in understanding the results from animal studies and especially in the translation of findings from animals to understanding human disease processes. Epidemiologic studies have the advantage of studying human populations with gene frequencies and exogenous exposure levels (e.g., nutrients) at the pertinent levels. However, there are other concerns with studies of this kind.

In epidemiologic studies, an individual's genotype can be determined from DNA extracted from cells. The DNA is typically obtained from blood cells, from sloughed cells in saliva, or from sloughed cells from the inside of the mouth. For polymorphic genes, laboratory methods are used to identify which of the variants for a particular gene are carried by an individual based on the sequence of the DNA. Because the presence of one mutation may be frequently found with the presence of others, it may not be necessary to measure every variant in a particular gene. Rather, markers or tag SNPs can be measured that identify the group of variants in a gene. These variant groups that tend to travel together are referred to as haplotypes.

An individual's phenotype may also be measured. Phenotype may be the measure of some physical characteristic such as body weight, eye color, or blood concentration of a vitamin. Alternatively, phenotype may be measured as the response to an exposure. An individual ingests a measured amount of a substance (e.g., caffeine), and then blood or urine samples are collected for several hours or days to determine the rate of metabolism of that substance. Measurement of phenotype is advantageous in that it provides an indication of the sum of the processes involved and therefore an indication of the true exposure. As noted previously, phenotype may depend on more than one gene; it may reflect processes involved in absorption and excretion as well as synthesis and metabolism. However, when comparisons are being made of individuals with and without a disease, the phenotype may be affected by the disease state and might not be reflective of the diseased individual's lifetime exposure. In studies that include people with disease, a determination of phenotype may be less useful than determination of a genotype, unless it can be shown that the phenotype does not change for those with the disease.

In epidemiology, the interaction of genes and diet may be assessed in case–control studies, in cohort and nested case–control studies, or in randomized trials. In case–control studies, the cases are the people with recently diagnosed disease and they are compared to healthy controls from the same population. Statistical methods are used to determine whether there are systematic differences between those people who get the disease and those who do not. In a prospective cohort study, a population of exposed and unexposed individuals is identified and their exposure status measured. Those individuals are then followed-up to compare the rate of disease in the exposed and unexposed groups. Because cancer is relatively rare, cohort studies generally need to include thousands or even hundreds of thousands of individuals in order to examine diet in relation to cancer.

When blood has been collected from study participants, it is possible to determine genotype for the participants. In a cohort study, a nested case–control study may be conducted for analysis of gene–environment interactions. Individuals in the cohort with incident cancer (cases) are compared to a group of non-cases selected from the cohort. The non-cases are chosen to be similar to the cases for relevant characteristics such as age, race, and gender. Genotypes are then determined and the gene–environment interactions analyzed for this subset of the whole cohort. Environmental exposures are estimated from interview data (e.g., a food frequency questionnaire) or from other, more direct measures of exposure (e.g., blood vitamin concentration).

In a randomized trial, study participants are randomly allocated to exposure category. For example, participants would be randomly selected into a group to be given a dietary intervention such as selenium. Others would be randomly selected to take a placebo. For randomized trials of supplements, often none of the participants know which group they are in. The trial is "blinded." For trials of change in diet, it is often not possible to blind the participants; they know if they are receiving the intervention diet or the control diet. To examine diet–gene interactions, the effect of the intervention could be compared by genotype. It is possible to determine genotype in advance of randomization in order to group participants based on their genotype, to ensure that the number of participants with a particular genotype is the same in the intervention and control groups.

For both case–control and nested case–control studies, the examination of gene–diet interactions generally involves a few steps: (1) the examination of the association between risk of a particular cancer and the genetic factor(s) alone—genetic factors can be a single polymorphism with known functional significance, haplotypes within a gene, a group of genes in one or more pathways, or thousands of variants across the entire genome; (2) the examination of the association between risk of that cancer and the dietary factor(s) alone—dietary factors can be nutrients, single food components, foods, groups of bioactive agents, or dietary patterns; and (3) the examination of the gene–diet interactions. In some cases, there will be further examination of interaction with other exposures (e.g., exposure to hormone replacement therapy or categories of body weight).

Although this is a relatively new area of research, in some clinical trials, the interaction of genes and environment is studied. The effect of a dietary intervention might be examined within and across groups to determine if one group is more susceptible to the intervention.

For all these study designs, there are some important methodological considerations. Many of these are the same considerations as for any epidemiologic study of diet and cancer [54]. A significant one is that, as in all epidemiology, conclusions about causality need to be derived from a synthesis of epidemiologic findings in a number of populations, as well as finding from animal research and metabolic studies. Generally, no single epidemiologic study can be considered definitive and used to establish causality. In case–control and cohort studies, there is concern that the findings may not be causal but rather the result of confounding. That is, another factor may be correlated both with the exposure under study and with the disease and may be the causative agent. If the investigators are unaware of this confounding factor or they are unable to control for it sufficiently well, it might appear that the exposure under study is associated with disease simply because it is correlated with the second factor. The confounding factor can be an exogenous exposure or another genetic factor. As defined above, sometimes certain genes tend to be inherited together and therefore one can confound an investigation of another of the genes, linkage disequilibrium.

Confounding may occur, for example, in a study of diet and lung cancer. In many populations, individuals who smoke are also more likely to drink alcohol. Unless smoking is measured well and controlled for in the analysis, one might incorrectly assess the relation of alcohol to lung cancer because of the correlation between these two behaviors. That is, it might be difficult to determine if alcohol is associated with lung cancer because of the confounding by smoking; disentangling the separate effects of alcohol from smoking may be very difficult. When studies are done in different populations in different cultures, the likelihood of confounding being the same in all of the populations is diminished; it is less likely that the same correlations exist between behaviors. In addition, other sources of error may differ among studies, making a consistent finding more believable. Only with a very well executed randomized trial can a causal link be identified with certainty because of the randomization to intervention or control group. However, even among randomized trials, differences in study results can occur because of differences in participant populations, so results need to be carefully interpreted. Clearly, it is only possible to stratify participants based on a limited number of genotypes, and linkage disequilibrium can make determination difficult as to which gene or genes are important in the process being studied.

Many hypotheses regarding gene–diet interactions in cancer risk have been examined in only a single study. With further development of the field, it will be possible to begin to identify the most consistent and important associations. At all times, it is important to examine findings critically and to search for consistent findings from well-conducted studies.

A major concern in evaluating diet and genetic interactions in relation to risk of cancer is that studies need to have large numbers of participants in order to have sufficient power to examine risk within strata defined by genotype or by diet. Because of the need to examine interactions, large studies are required to provide a sufficient number of individuals within the group of interest. Even if the results are statistically significant, such findings can be unstable. That is, if the study were redone, findings may not be consistent. In particular, for genes with low frequencies, the number of participants required to obtain a stable result can be very large. Furthermore, to examine the interactions of more than one gene and more than one dietary or other exposure, the number of participants required may be exceedingly large. In the evaluation of any study, it is important to consider the number of participants in each cell to make a determination of the likely stability of the findings.

Cancer is a multifactorial disease, and it is likely that several genetic factors are of importance even within a single causative pathway. Analysis of a single polymorphism or even of several genes in a pathway might not capture the total picture of variation in risk. Similarly, several nutrients are likely to be important even within a single causal pathway. As noted previously, the examination of gene–gene and gene–environment interactions can be seriously hampered by the required sample size.

Diet is also a complex set of exposures. A single food may include different factors with both carcinogenic and anticarcinogenic properties. Intakes of different nutrients are often highly correlated so that an association attributed to intake of one nutrient may in fact be the result of a causal relationship with a different nutrient. These differences are very important if the findings are interpreted to mean that

taking a supplement of a single nutrient will have a protective effect. Finally, when there is evidence that a particular nutrient is related to a decrease in risk, it cannot be assumed that larger quantities of that nutrient would be even more protective. For example, whereas vitamin C has antioxidant properties at the level found in most foods, at higher intake levels, it may have pro-oxidant properties [55].

A final challenge in this field is in the measurement of dietary exposure. Much literature is available on the problems of measurement of diet for epidemiologic purposes [56]. Beyond those concerns, the study of gene–diet interactions in relation to cancer risk has led to an interest in a number of new bioactive compounds found in foods. For many of these compounds, the dietary instruments used in the past may not provide sufficient detail for assessment of intake of that compound. For example, the study of heterocyclic amines led to the development of a questionnaire that specifically addresses the sources of these compounds because the information regarding intake on the existing questionnaires did not include the necessary detail to assess intake of heterocyclic amines [57]. Furthermore, nutrient composition databases may be limited and may need work in order to determine the composition for compounds that may relate to disease risk but that have not been previously analyzed.

V DIET–GENE INTERACTIONS AND CANCER

As described previously, there are a number of properties that distinguish a tumor. These properties contribute to the ability of a tumor to break loose from normal control of cell growth and to invade tissues. Tumor cells have their own growth signals, they do not respond to signals inhibiting growth, they can replicate indefinitely and do not go into programmed cell death (apoptosis), they can invade other tissues (metastasis), and they can develop a blood supply for those metastases (angiogenesis) [7]. All of these pathways may be appropriate targets for cancer prevention. Given the nature of tumor biology, it is unlikely that there will be one food or compound that will prevent all cancers or even all of one kind of tumor; rather, the cumulative effect of a number of compounds may be important in prevention [58]. Many processes are important in carcinogenesis, including carcinogen metabolism, hormone regulation, cell differentiation, DNA repair, apoptosis, cell growth cycle, and inflammatory response. Nutrients and other bioactive food components may play a role in each of these processes (Fig. 33.7) [59]. This area of research is rapidly changing, and our understanding of these relationships is likely to shift with further investigation. This overview is provided to give examples of the developing understanding.

A Carcinogen Metabolism

One example of a possible interaction of genetic factors with diet is the association of alcohol and esophageal cancer. There is consistent epidemiologic evidence that heavy alcohol consumption is associated with increased esophageal cancer risk [60]. There is also emerging evidence of increased risk even among light drinkers, especially in Asian countries [61]. Although there are several possible mechanisms to explain these observations, it may be that the mechanism of this association includes gene–environment interactions in carcinogen metabolism. As described earlier, the rate of metabolism of alcohol to acetaldehyde is impacted by an SNP, which is common among Asians. Acetaldehyde has been identified as a carcinogen [60]. It may be that among light drinkers, the exposure to alcohol combined with slower metabolism in those Asians with the gene for slower metabolism accounts for the increased risk associated with alcohol consumption.

B Regulation of Hormones

There is considerable evidence that estrogen exposure is a strong risk factor for breast cancer [62]. Of interest is whether compounds of plant origins with estrogenic properties, called phytoestrogens, affect breast cancer risk. Among these is indole 3-carbinol, which is found in cruciferous vegetables after they are cooked or crushed. There is evidence from studies of breast cells in culture that this compound can affect the estrogen receptor, ER-α. It can alter the estrogen and the DNA binding regions of the ER, leading to decreased response to estrogen in estrogen-responsive cells [63]. Thus, the interaction of this dietary compound with DNA may affect breast cancer risk because of the dampening of response to estrogen. Another phytoestrogen is lignin, found in flax seed, whole grains, sesame and sunflower seeds as well as smaller amounts in some fruits and vegetables. Increased lignin intake has been found to be associated with lower breast cancer risk [64].

C Cellular Differentiation

Genistein, a polyphenol in soy, has been found to decrease mammary tumors in animal models. There is evidence that when genistein is ingested in the prepubertal rat, it can help to regulate mammary development and differentiation. An increase in differentiation of mammary tissue is observed, as are differences in cellular proliferation, apoptosis, and tumor suppressor expression. The effects of genistein on mammary development appear to be most beneficial with administration of this bioactive compound in young animals and may not have the same impact in older ones [65].

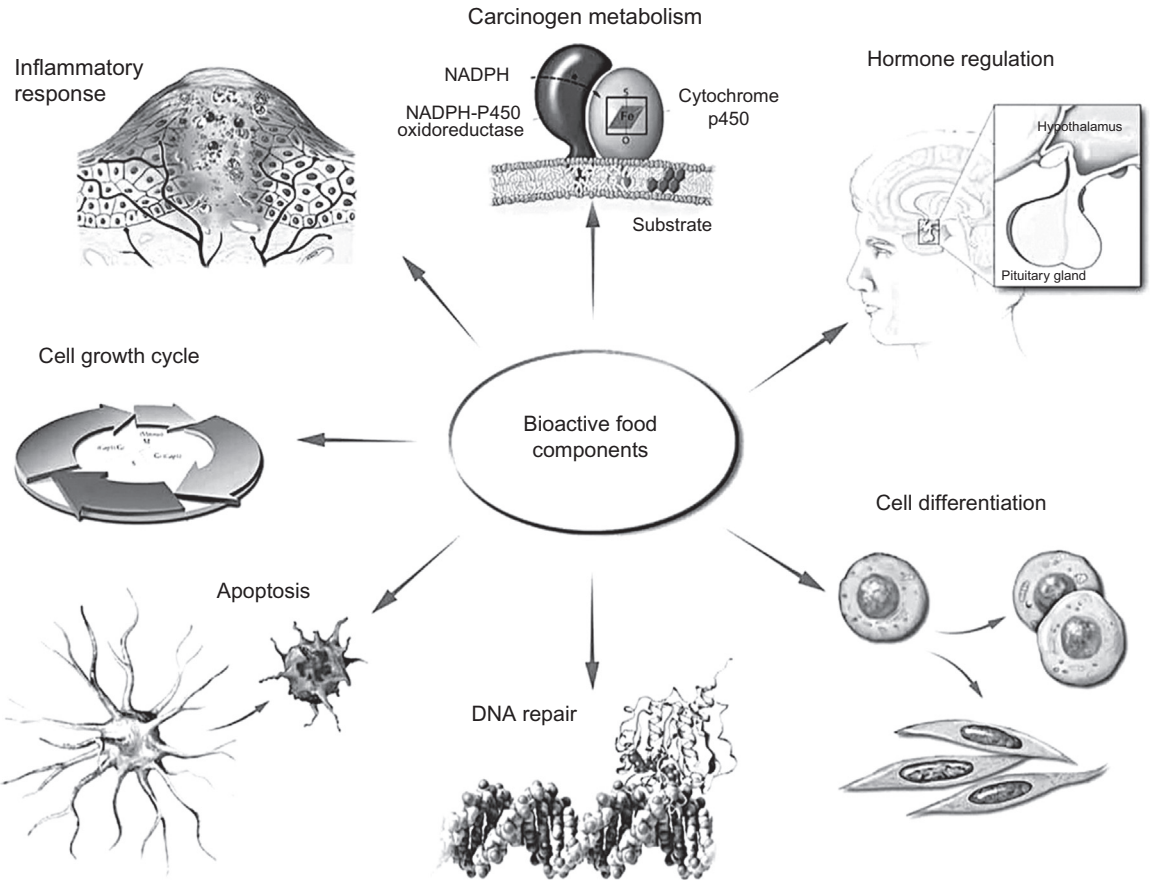

FIGURE 33.7 Interactions of bioactive food components with genes appear to be important mechanisms for carcinogenesis. *From E. Trujillo, C. Davis, J. Milner, Nutrigenomics, proteomics, metabolomics and the practice of dietetics, J. Am. Diet. Assoc. 106 (2006) 403–413. Copyright The American Dietetic Association. Reprinted by permission.*

D MicroRNAs

MicroRNAs (miRNAs) are noncoding RNA molecules that regulate genes by controlling the translation and degradation of messenger RNA. A single miRNA can impact more than one gene and a single messenger RNA can be regulated by more than one miRNA. There is emerging evidence that dietary factors affect miRNAs. For example, there is evidence of curcumin from cumin, green tea catechins, and retinoic acid, all affecting particular miRNAs in cells in culture. It is possible that these effects in an organism could alter biological processes significant in carcinogenesis including apoptosis, proliferation, and cell growth [66].

E Apoptosis

Programmed cell death or apoptosis is an important function in the maintenance of the integrity of tissues. Dietary factors may also affect apoptosis and may interact with genes that control it. For example, there is evidence that vitamin D can influence genes controlling apoptosis [67]. Other dietary factors may also play a role. In one study, overweight or obese postmenopausal women were placed on a low-fat, high-fiber diet, and an exercise program. A comparison was made of the growth-promoting properties of serum from the women taken before and after the intervention. The properties were evaluated in three different estrogen receptor-positive breast cancer cell lines. Among other changes, there was an increase in apoptosis for several different cell lines when the postintervention serum was used as the growth medium [68]. Other dietary factors that may also affect the expression of genes important in apoptosis include organosulfur compounds from foods from the allium family, including garlic; polyphenols from green tea, chocolate, and chili peppers; and isothiocyanates from cruciferous vegetables [69].

VI FUTURE DIRECTIONS

Understanding of the role of genes in cancer and particularly the role of the interactions of diet with genes in this

disease process is a rapidly expanding field of inquiry. Most of the available evidence remains somewhat preliminary, based on cell culture and animal studies or on a small number of epidemiologic studies. Our understanding of gene−diet interactions and how to study them is advancing rapidly, with tremendous potential implications for our understanding of the role of dietary factors in cancer etiology. Clearly, as the field progresses, extremely large studies will be needed to allow for the examination of multiple levels of interaction to fully understand the relevant etiological pathways.

In the future, genotype needs to be examined in metabolic studies to determine the short-term effects on intermediate outcome measures in healthy individuals. A great deal remains to be understood about the relation between genotype and phenotype for the genes that appear to be important in cancer risk. Furthermore, we need to understand the exposures—both dietary and from other sources—that affect gene expression in the most important etiological pathways. More work must be performed to identify the relevant genes that make the most difference, possibly those that are control points for metabolic pathways or for other pathways of significance. It will also be important to identify variants that are sufficiently common in the population to have an impact. We are moving toward the examination of the role of diet in cancer etiology, examining effects within groups defined by genetic makeup. Such studies may allow us to identify relationships that are important among particular groups of the population but that are not evident when the whole population is studied together.

There is increasing evidence that exposures in early life may be important in the carcinogenic process for some cancers. For example, there is evidence of associations of decreased risk of adult breast cancer with higher consumption of dietary fiber, vegetable protein, and vegetable fat during adolescence [70]. Some genetic factors may be more important in those early years such that studies examining adult diet do not provide the whole picture of the interaction of a nutrient with a genetic factor in cancer risk. Research needs to be done regarding such interactions.

There is also accumulating evidence that diet may affect prognosis following a cancer diagnosis. There is considerable evidence, for example, that weight gain after breast cancer diagnosis is associated with poorer survival [71]. The factors affecting this association are not well understood. It is not known if there are different kinds of weight gain which affect prognosis differently. In addition, it is not known if genetic factors interact with the added weight and impact mortality. In addition to weight gain, it could be that other nutritional factors affect cancer recurrence or progression and that the effects vary depending on the individual's genetics as well as the genetic mutations in the tumor.

There is little doubt that environmental factors are very important in causing cancer. Genetic factors also contribute to the processes determining who will get a cancer and who will not for most cancers. Important among the likely environmental factors are dietary factors. With increased understanding of the interactions of genetic and nutritional factors, it may eventually become possible to identify individuals with higher or lower requirements for particular nutrients or to identify individuals with greater sensitivity to agents such as alcohol. Furthermore, the elucidation of genetic factors in relation to diet will help us to truly understand the natural history of cancer and how best to prevent it.

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Chapter 34

Nutrition and Cancers of the Breast, Endometrium, and Ovary

Kim Robien[1], Cheryl L. Rock[2] and Wendy Demark-Wahnefried[3]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*University of California at San Diego, La Jolla, CA, United States*

[3]*University of Alabama at Birmingham, Birmingham, AL, United States*

I INTRODUCTION

Carcinomas of the breast, endometrium, and ovary are hormone-related cancers that have biologic similarities. Among U.S. women, breast cancer is far more common than endometrial or ovarian cancers. In 2011, roughly 230,480 women were diagnosed with breast cancer, thus comprising approximately 30% of the incident cancers among females [1]. In contrast, cancers of the endometrium (uterine corpus) and ovary comprised approximately 6% and 3% of female cancers, respectively. Because of differences in treatment efficacy and usual stage at diagnosis, the mortality estimates for these cancers vary considerably. Breast cancer will be the cause of death for approximately 39,520 women during 2011 (approximately 15% of cancer-related deaths among U.S. females), whereas endometrial and ovarian cancers account for approximately 3% and 6% of cancer-related mortality among women, respectively [1].

Breast cancer occurs infrequently in men, although the incidence is rising. In 2011, an estimated 2140 men were diagnosed with breast cancer, and 450 men will die of this disease [1], accounting for more than 1% of all malignancies and cancer deaths in men. Men tend to have a less favorable breast cancer outcome compared to women, largely due to advanced stage at diagnosis. More than 40% of male breast cancer patients present with stage III or IV disease [2].

Estrogens likely play an important role in breast, endometrial, and ovarian cancers [3]. Normal cell proliferation and differentiation in these tissues is highly responsive to estrogens and the other gonadal hormones. In addition to the ovarian steroids, other growth factors and mitogens influenced by nutritional factors and dietary patterns also appear to play an important role in the initiation and promotion of breast cancer. Insulin and insulin-like growth factor 1 (IGF-1) are currently under active investigation, as well as the interactions of these factors with adiposity and weight gain [4].

Diet and/or nutritional status is presumed to play a major role in the risk and progression of these hormone-related cancers, either through influence on the hormonal milieu or gene expression or via direct effects. Compared to the amount of research on breast cancer, far fewer studies have examined the relationship between nutritional factors and risk and/or progression of endometrial and ovarian cancers. This chapter reviews and summarizes evidence on the relationships between nutritional factors and breast, endometrial, and ovarian cancers. Recent clinical and epidemiological studies are emphasized, with the goal of identifying clinically useful strategies for prevention and patient management.

II BREAST CANCER

A Nutritional Factors and Primary Breast Cancer Risk

1 Height, Weight, and Body Fat Distribution

During the past four decades, numerous studies have evaluated the association between height and breast cancer risk, including a meta-analysis by the World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) confirming a strong association for both pre- and postmenopausal disease [5]. Furthermore, height

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has also been associated with increased mammographic density, which is considered a strong risk factor for breast cancer [6]. It is hypothesized that the observed associations between height and breast cancer are due to factors that promote growth rather than height. Height attainment during puberty and selected periods during childhood has also been investigated in relation to breast cancer risk [7,8]. Other studies have concentrated on birth size as a proxy for in utero exposure. A meta-analysis (17 cohort and 15 case−control) found a statistically significant increased risk of breast cancer among women who weighed 4.0 kg or more at birth (relative risk, RR = 1.12; 95% confidence interval (CI), 1.00−1.25) compared to women with birth weights between 3.0 and 3.5 kg [9]. Birth length and head circumference obtained from birth record data were also found to be positively associated with breast cancer risk [9]. Other skeletal indices, such as increased elbow breadth (a frame size marker), femur and trunk length, and bone density, have also been associated with increased risk [10−12].

A clear discrepancy exists between adiposity and its apparent effect on risk for pre- versus postmenopausal disease. Leanness is an acknowledged risk factor for premenopausal breast cancer, whereas obesity serves as a strong risk factor for postmenopausal disease [5]. A meta-analysis of cohort studies reported a 15% decreased risk of premenopausal breast cancer and an 8% increased risk of postmenopausal breast cancer per 5 kg/m^2 difference in body mass index (BMI) [13]. These risks are modified by (1) relative chronicity within the broad categories of pre- versus postmenopause (i.e., younger obese postmenopausal women may be at greatest risk [14]), (2) hormone-replacement therapy use (which may mask associations between obesity and postmenopausal disease) [14,15], and (3) genetic variation [16,17]. Studies also indicate that obesity is a strong risk factor only for estrogen receptor (ER) and progesterone receptor (PR) positive breast cancer [18,19]. The relationship between obesity and breast cancer risk in men is similar to the relationship observed in postmenopausal breast cancer in women [20,21].

There are several hypothesized mechanisms by which overweight and obesity confer risk for male and postmenopausal female breast cancer [22]. A classic hypothesis involves the increased peripheral aromatization of androgens within the adipose tissue, thus yielding increased levels of circulating estrogens. Studies show that obese women have significantly higher circulating levels of total testosterone, estrone, and estradiol, as well as higher free levels of these hormones [23,24], thus providing support for this premise.

Obesity also results in increased circulating levels of insulin and IGFs, which stimulate intracellular signaling pathways and enhance proliferation of cancer cells [25]. For premenopausal breast cancer, in which increased skeletal structure is associated with risk, the IGF-1 pathway has also been implicated as a potentially viable mechanism [26]. Studies are ongoing to determine the role of leptin, adiponectin, interleukin-6, tumor necrosis factor, and other potential mediating factors that may have an impact on carcinogenesis via inflammation [27].

Diabetes has been associated with risk of many cancers, including postmenopausal breast cancer, although the exact mechanisms are unclear [28]. In a prospective analysis of 12,792 cancer-free participants in the Atherosclerosis Risk in Communities study, nondiabetic women with elevated glycated hemoglobin levels (hazard ratio, HR = 1.24; 95% CI, 1.07−1.44) and diabetic women (HR = 1.30; 95% CI, 1.06−1.60) were at increased risk of breast cancer compared to nondiabetic women with normal glycated hemoglobin levels [29]. Similarly, in the Women's Health Initiative (WHI) Observational Study, fasting insulin levels were found to be positively associated with breast cancer risk (HR = 1.46; 95% CI, 1.00−2.13 for highest vs lowest quartile of insulin level; p-trend = 0.02) [30]. The observation that cancer incidence is lower among individuals with diabetes taking metformin [31], which suppresses hepatic gluconeogenesis and increases insulin sensitivity in peripheral tissues, has led to a number of clinical trials of metformin as a breast cancer chemopreventive agent that are currently underway [25].

Because body weight fluctuates throughout life, it is conceivable that risk may be modified by body weight status at differing ages. Birth weight greater than 4000 g was associated with a small but statistically significant increased risk of breast cancer compared to birth weights between 2500 and 2599 g (odds ratio, OR = 1.24; 95% CI, 1.04−1.48) [32]. Other studies that have explored obesity during childhood suggest that it may be protective [33,34], with one study suggesting that this relationship is particularly significant among females with a positive family history of breast cancer [35]. An analysis from the Nurses' Health Study (NHS) II [36] suggests that an increased BMI at age 18 years may be the strongest protective factor for premenopausal disease. A possible explanation for this unanticipated finding is that obese teenage girls have significantly fewer ovulatory cycles and therefore lower circulating levels of both estrogen and progesterone [37]. Weight gain and increased body weight in adulthood have consistently been found to be associated with increased risk for postmenopausal cancer [38−40], although risk may be altered by use of hormone therapy and ethnicity [41,42]. Data from the European Prospective Investigation into Cancer (EPIC) study indicate an 8% increase in risk for each 5-kg gain among nonhormone therapy users [41], which may be largely explained by the fact that as women age, their circulating levels of estrogen become more influenced by estrogens produced by adipose tissue than those produced by the

ovary [22]. Weight gain during adulthood is also more likely to be deposited in an android versus gynoid pattern and hence may promote insulin resistance and the increased production of insulin and IGFs [43]. A meta-analysis (three cohort and eight case−control studies) found that adult weight gain was associated with statistically significant increased risk of both ER^+/PR^+ and ER^-/PR^- breast cancers, although the risk was higher for ER^+/PR^+ breast cancers (summarized risk estimate, RE = 2.03; 95% CI, 1.62−2.45) than ER^-/PR^- tumors (RE = 1.34; 95% CI, 1.06−1.63) [44].

In contrast, studies that have investigated weight loss have found a protective effect [45]. Parker and Folsom [46] found a risk reduction for postmenopausal breast cancer of approximately 19% among women who intentionally lost 20 pounds or more during adulthood. Among women carrying the BRCA1 mutation, weight loss of 10 pounds or more between the ages of 18 and 30 years was associated with a risk reduction of approximately 50% [47].

Studies consistently show that abdominal obesity (primarily assessed via waist:hip ratio) is associated with increased risk of postmenopausal breast cancer; however, a 2007 meta-analysis by the WCRF/AICR found that these associations disappear when analyses are controlled for BMI [13]. Associations between central obesity and increased risk may still exist for select populations, such as Asians [48].

2 Dietary Composition

In ecologic studies, a fivefold difference in breast cancer mortality rates has been observed across countries, and dietary patterns may be one of the environmental exposures that differ across these countries [49]. Also, risk for breast cancer increases on relocation from low-risk countries to high-risk countries, concurrent with the adoption of the dietary and lifestyle patterns of the new locale [50].

The possible link between dietary fat intake and breast cancer risk has received the most attention. However, fat intake, total energy consumption, and adiposity are inextricably linked; therefore, demonstrating an independent effect of total dietary fat per se is a challenge [51]. A meta-analysis of data from five cohort studies showed no statistically significant association between dietary fat and breast cancer risk (RR = 1.06; 95% CI, 0.99−1.14) [13].

Various feeding studies and small diet intervention trials have examined the effect of dietary fat reduction on serum estrogen levels. Low-fat diets were associated with an average 13% reduction in serum estradiol concentrations in a meta-analysis of several small feeding studies [52]. However, significant weight loss occurred in most studies in which serum estradiol was significantly reduced, and dietary fiber was also concurrently increased in 8 of the 13 studies included in the analysis. Thus, an energy deficit, weight loss, or increased fiber intake are just as likely as fat to promote a reduction in hormone levels.

In the WHI randomized controlled dietary modification trial, the impact of a low-fat diet (<20% of energy intake) was tested in comparison to a usual case−control group as a potential means of reducing risk of breast cancer in 48,835 postmenopausal women during an 8-year follow-up period [53]. Adherence was an issue in both study arms, with those in the intervention arm having higher fat intakes than planned, and those in the control arm reporting reductions in intake such that the difference in fat intake between the two study arms was not as great as anticipated (i.e., a difference of only 10.7% in year 1 and 8.1% in year 2). Results indicate a nonsignificant impact on risk (HR = 0.91; 95% CI, 0.83−1.01). Similarly, the Canadian Diet and Breast Cancer Prevention Trial, a multicenter randomized controlled study that tested whether a low-fat (15% of energy), high-carbohydrate diet intervention could reduce the incidence of breast cancer among women with extensive mammographic density, found that a sustained reduction in dietary fat intake did not reduce breast cancer risk, even though the intervention group was able to decrease dietary fat intake from 30% to 20% of total energy intake and maintained a dietary fat intake that was 9 or 10% lower than that of the comparison group throughout the study [54].

The effect of dietary carbohydrates on breast cancer risk has also been evaluated. A meta-analysis of eight cohort studies found no association between glycemic index (GI) (RR = 1.06; 95% CI, 0.98−1.15 for highest vs lowest subgroup) or glycemic load (GL) (RR = 0.99; 95% CI, 0.94−1.06 for highest vs lowest subgroup) and breast cancer risk [55]. A second systematic review and meta-analysis published the same year also found no statistically significant associations between GI or GL and breast cancer risk among pre- or postmenopausal women [56]. Despite the significant limitations of GI and GL determinations in general [57], these findings suggest that weight management may be more effective in decreasing breast cancer risk than modifying dietary carbohydrate intake.

Dietary fiber has been hypothesized to exert a protective effect against breast cancer by binding estrogen in the enterohepatic circulation and hindering reabsorption [58]. However, studies that have examined the relationship between fiber intake and risk for breast cancer have generally not found a significant protective effect [13].

Several studies have examined the association between vegetable and fruit intake and breast cancer risk. A meta-analysis (21 case−control and 5 cohort studies) found that high (vs low) consumption of vegetables exhibited a significant protective effect (RR = 0.75; 95% CI, 0.66−0.85), whereas fruit consumption was not associated with breast cancer risk [59]. A subsequent pooled analysis

including 7377 incident breast cancer cases from 8 prospective cohort studies found a small and only marginally significant protective effect of total fruit and vegetable intake (RR = 0.93; 95% CI, 0.86−1.00 for highest vs lowest quintiles) [60]. Although the median period of follow-up was only 5.3 years, the relationship between vegetable and fruit intake and breast cancer risk also was not significant in the EPIC study [61].

Studies in which tissue concentrations of carotenoids (a marker of vegetable and fruit intake) have been quantified and analyzed suggest a protective effect of higher concentrations of these compounds. In a report from the New York University Women's Health Study [62], α- and β-carotene, lutein, and β-cryptoxanthin levels were inversely associated with breast cancer risk. Similarly, an analysis from the WHI using repeated measures of serum carotenoids, retinol, and tocopherols at years 1, 3, and 6 of the study also found that breast cancer risk was inversely associated with serum α- and β-carotene levels but positively associated with γ-tocopherol levels [63]. In a pooled analysis of 18 prospective cohort studies, α- and β-carotene and lutein/zeaxanthin intakes were inversely associated with risk of ER^- breast cancers but not ER^+ breast cancers [64].

Cell culture studies indicate that retinoids and carotenoids affect cellular differentiation and also inhibit mammary cell growth [65]. Antioxidants, such as vitamin C, may reduce the risk for breast cancer by protecting against DNA damage and other free radical−induced cellular changes associated with neoplasia. Vegetables of the *Brassica* genus, such as broccoli, may favorably alter estrogen metabolism via the induction of cytochrome P450 enzymes [66].

Countries that consume greater amounts of soy and soy products, mainly Asian countries, have historically exhibited the lowest breast cancer mortality rates, compared with the United States and most European countries [67]. Thus, the role of soy and soy isoflavones in breast cancer prevention has been investigated in numerous laboratory and epidemiological studies, but the findings remain inconclusive [13]. Soy is a rich source of phytoestrogens; however, they can act as both estrogen agonists and antagonists [68]. The inconsistent findings between Asian and non-Hispanic white study populations suggest that lifelong or early life soy intake may be necessary in order to observe the breast cancer protective effects [69].

Alcohol intake has been consistently and positively associated with breast cancer risk in epidemiological studies. Pooled analysis of data from six cohort studies indicates that alcohol exhibits a dose−response relationship with risk for breast cancer, at least up to 60 g/day (RR = 1.41; 95% CI, 1.18−1.69, for 30−60 g/day vs no alcohol) [70]. Another meta-analysis confirmed this finding of an increased risk for breast cancer overall (RR = 1.10; 95% CI, 1.06−1.14, per 10 g alcohol/day), and when stratified by premenopausal (RR = 1.09; 95% CI, 1.01−1.17) and postmenopausal (RR = 1.08; 95% CI, 1.05−1.11) breast cancer [13]. The proposed biologic mechanism is that alcohol intake promotes increased serum estrogen levels, which then increase breast cancer risk. Studies of other beverages, such as coffee and tea, have found no association with breast cancer risk [13,71,72].

Although an association between folate intake per se and breast cancer risk has not been observed consistently in epidemiological studies, results from several cohort studies suggest that dietary folate intake may influence breast cancer risk through an interaction with alcohol [73−75]. Alcohol is known to interfere with folate metabolism [76]. A meta-analysis of two prospective cohort studies and two case−control studies found that high dietary folate intake (compared to low folate intake) was protective against breast cancer among women who had moderate to high alcohol intake (summary estimate = 0.51; 95% CI, 0.41−0.63) but not among women who reported low or no alcohol intake [75].

Vitamin D is known to have antiproliferative activity in a variety of cell types [77] and thus may decrease cancer risk. Prospective cohort studies consistently report decreased risks of breast cancer with increased vitamin D intake despite the relatively low intake of dietary vitamin D of the populations studied [78]. However, results vary across studies depending on the menopausal status of the participants and tumor characteristics. The NHS [79] observed decreasing incidence of breast cancer with increased intake of dietary or supplemental vitamin D, but only among premenopausal women (RR = 0.72; 95% CI, 0.55−0.94). A report based on the Women's Health Study also found reduced risk of breast cancer with total vitamin D at levels beginning above 230 IU among pre- but not postmenopausal women [80]. Results were stronger for premenopausal women with ER^+/PR^+ or more advanced stage tumors. In the Cancer Prevention Study (CPS) II Nutrition Cohort of postmenopausal women [81], a weak inverse association was observed between dietary vitamin D and breast cancer (RR = 0.87; 95% CI, 0.75−1.00, comparing >300 IU to ≤100 IU/day). However, the vitamin D associations were somewhat stronger for women diagnosed with ER^+ tumors. Among postmenopausal women participating in the Iowa Women's Health Study (IWHS) [82], those consuming ≥800 IU of vitamin D from diet and supplements had an 11% reduced risk of breast cancer. Findings were stronger for women with ER^- or in situ tumors.

Because diet is a complex, multidimensional, chronic exposure [83], there has been interest in evaluating dietary patterns and adherence scores for dietary

recommendations as measures of overall diet quality. In an analysis of the NHS data, compliance with the Dietary Approaches to Stop Hypertension (DASH) diet was inversely related to risk of ER^- breast cancers, but no association was observed for risk of ER^+ tumors [84]. Similarly, a dietary pattern high in fruits and salads was associated with a reduced risk of breast cancer, especially ER^- and PR^- breast cancers, among participants in the Melbourne Collaborative Cohort Study [85]. Among Chinese women participating in the Shanghai Breast Cancer Study, a Western diet pattern ("meat–sweet" pattern: shrimp, chicken, beef, pork, candy, and desserts) was associated with an increased risk of breast cancer in postmenopausal women, especially with ER^+ tumors [86].

To date, only one small case–control study has explored the association between intake of selected foods and male breast cancer [87]. No statistically significant associations were reported; however, study design issues (e.g., small sample size, use of death index to identify cases, and use of next-of-kin interviews to assess dietary intake among cases) limit the ability to draw conclusions from this study, and further research is needed.

B Nutritional Factors and Breast Cancer Recurrence, Progression, and Survival

Breast cancer mortality has been declining in recent years—a trend that has been attributed to earlier diagnosis and improvements in treatment [1]. A majority of all breast cancers are now diagnosed at a localized stage, with 98% 5-year relative survival rates [1]. As a result, there are increasing numbers of women in the population who are breast cancer survivors and at risk for breast cancer recurrence. Women who have been diagnosed with breast cancer have higher rates of mortality from other causes, such as cardiovascular disease and diabetes—comorbid factors in which diet and nutritional status play an important role [88].

1 Obesity and Overweight

Weight is the nutritional factor associated most consistently with mortality. Although several studies suggest that overweight and obesity may be linked to increased recurrence or to progressive disease [89–92], findings are somewhat mixed with regard to these outcomes [93–95]. However, because overweight is associated with several other chronic diseases, data are fairly consistent regarding overall survival, for which normal-weight women demonstrate a significant advantage [96]. Findings by Pierce and colleagues [97] suggest that physical activity may be more important than weight in conferring a survival advantage; however, further study is needed. Compared to those who are overweight, normal-weight women experience significantly fewer surgical complications [98,99], less lymphedema [100,101], and fewer thromboembolic events while on hormonal therapy [102]. Studies exploring body fat distribution in relation to survival or comorbid conditions have yielded mixed findings [96,103–105].

Weight gain after the diagnosis of breast cancer is a common occurrence and may be undesirable for several reasons. First, weight gain may negatively affect quality of life [106]. Second, weight gain may predispose women to other chronic conditions, such as hypertension, cardiovascular disease, diabetes, and impaired mobility [107]. Finally, weight gain may adversely affect disease-free survival. Kroenke and colleagues [108] found that breast cancer survivors who increased their BMI by 0.5–2 units had an RR of recurrence of 1.40 (95% CI, 1.02–1.92), and those who gained more than 2.0 BMI units had an RR of 1.53 (95% CI, 1.54–2.34); both groups also experienced significantly higher all-cause mortality. In contrast, Caan and colleagues [93] found no evidence of increased mortality with weight gain in a cohort of 3215 early-stage breast cancer patients. More study is needed in this area, especially to determine the impact of weight-loss interventions on survival and other short- and long-term outcomes.

Reports of mean weight gain during chemotherapy vary considerably but typically range from 1 to 5 kg [109]. Some have reported that as many as 20% of women gain 5 kg or more [110]. Weight gain is most prevalent among premenopausal patients receiving adjuvant chemotherapy and may vary by treatment regimen, with anthracycline agents associated with greater gains [109,111,112]. Several groups have conducted weight-loss interventions among women with breast cancer, with success rates differing by type and duration of intervention. Differential effects were found for individual diet counseling by a dietitian, with one study showing significantly favorable effects on body weight status [113] and the other showing no effect [114]. Djuric and colleagues [115] found that counseling by a dietitian was most effective if combined with a structured group weight-loss program that included exercise. Behavioral interventions that utilize a comprehensive approach to energy balance by including both diet and exercise components may be more effective than interventions relying on either component alone. Goodwin et al. [116] found that exercise was the strongest predictor of weight loss among early-stage breast cancer patients receiving a diet and exercise intervention during the time of treatment and extended throughout the year following diagnosis. Exercise (especially strength training exercise) may be of particular importance for cancer survivors because it is considered the cornerstone of treatment for sarcopenic obesity (gain

of adipose tissue at the expense of lean body mass), a documented side effect of both chemotherapy and hormonal therapy [111].

2 Dietary Composition

Observational studies of the relationship between fat intake and survival after a breast cancer diagnosis have reported inconsistent findings, with some studies reporting inverse associations between survival and dietary fat intake at diagnosis [117–119], whereas others [120–122] have found no association.

Protective effects of vegetables and fruits and the micronutrients provided by these foods (e.g., vitamin C and carotenoids) have been observed in several of these cohort studies, with findings somewhat more consistent than those for dietary fat, although the strength of the association is modest. Several studies reported significant inverse associations between fruit and vegetable intake and risk of death [117,118], two found that risk of dying was nonsignificantly decreased in association with frequent vegetable consumption [123,124], and one found a significant inverse association in women with node-negative disease, who comprised 62% of that cohort (but not in the total group, which included women at all stages of invasive breast cancer) [121]. In the studies that found an inverse relationship with survival and intakes of vegetables, fruit, and related nutrients (β-carotene and vitamin C), the magnitude of the protective effect was a 20–90% reduction in risk for death. In a cohort study involving 1511 women previously diagnosed and treated for breast cancer, women in the highest quartile of plasma total carotenoid concentration had an estimated 43% reduction in risk for a new breast cancer event (recurrence or new primary) compared to women in the lowest quintile [125]. In the same cohort, breast cancer survivors who reported consuming five or more daily servings of vegetables and fruit and exercised at least at a moderate level for 30 minutes nearly every day had a 50% reduction in risk associated with these healthy lifestyles [97]. An overall dietary pattern characterized by higher intakes of vegetables, fruit, whole grains, and low-fat dairy products was not related to all-cause or breast cancer mortality but was related to a significantly lower risk of mortality from other causes during a 20-year follow-up period in another cohort of 2619 women [126].

Although alcohol intake has been identified as a risk factor for primary breast cancer, the effect on breast cancer recurrence or survival is unclear, with studies reporting increased [127–130] or decreased [97,119,131,132] risk of death, as well as no association [121,133,134]. The Collaborative Women's Longevity Study ($N = 4441$) found a statistically significant trend toward lower risk of death with higher alcohol consumption (p-trend = 0.01) but no association with breast cancer–specific survival [119]. In contrast, the Life after Cancer Epidemiology (LACE) study ($N = 1897$) found that consuming three or more alcoholic drinks per week was associated with increased risk of breast cancer recurrence, especially among postmenopausal and overweight/obese women [130]. One study ($N = 472$ with a history of early-stage breast cancer) found that beer intake was directly related to risk for recurrence (but not survival), and wine and hard liquor intake were unrelated to risk for either outcome [127].

Two multicenter randomized controlled intervention trials have tested whether diet modification can influence the risk for recurrence and overall survival following the diagnosis of early-stage breast cancer. In the Women's Intervention Nutrition Study (WINS), which involved 2437 postmenopausal women randomized within 12 months of primary surgery, the primary dietary goal was a reduction in dietary fat intake (<15% energy from fat) [135]. Women in the intervention arm in WINS reported a reduction in fat intake (33.3 g/day vs 51.3 g/day in the control group), which was associated with an average 6-pound weight loss. The HR of relapse events in the intervention group compared with the control group was 0.76 (95% CI, 0.60–0.98) after approximately 5 years of follow-up [136]. Results of secondary analysis suggest a greater protective effect among women with hormone receptor-negative breast cancers compared with women whose cancers were hormone receptor-positive. In the Women's Healthy Eating and Living (WHEL) study, the target population consisted of 3088 pre- and postmenopausal women who had been diagnosed with breast cancer within the preceding 4 years and who had completed initial therapies. The primary emphasis of the WHEL study diet intervention was on increased vegetable and fruit intake, with daily dietary goals of five vegetable servings, 16 ounces of vegetable juice or equivalent, three fruit servings, 15–20% energy intake from fat, and 30 g dietary fiber. Feasibility study reports and trial data from this study indicated excellent adherence [137,138], and increased intake of vegetables and fruit in the intensive intervention group was validated by plasma carotenoid concentrations [139]. Overall findings indicated no significant differences in risks for recurrence or survival in the intensive intervention versus the control group during a mean 7.3-year follow-up [140]. Notably, the study participants reported at baseline an average daily consumption of 7.3 servings of vegetables and fruit, which actually meets or exceeds current recommendations, so the WHEL study results mainly indicate that an extraordinarily high intake of vegetables and fruit does not appear to further reduce risk for recurrence.

The effect of dietary patterns on breast cancer recurrence and survival has been evaluated in several large

cancer survivor cohorts. Higher adherence to a prudent diet pattern (high intakes of fruits, vegetables, whole grains, and poultry) was not associated with recurrence but was associated with significantly improved survival among participants in the LACE study [141]. In the NHS, higher intake of a prudent dietary pattern and lower intake of a Western dietary pattern was associated with lower risk of death unrelated to breast cancer [126]. These findings are not surprising given the known beneficial effects of the prudent diet pattern in the prevention of chronic disease, especially cardiovascular disease, for which breast cancer survivors are at increased risk [88].

III ENDOMETRIAL CANCER

Cancer of the endometrium (uterine corpus) is the most common invasive gynecologic cancer [142]. Similar to breast and ovarian cancers, endometrial cancer is most common after menopause and is more prevalent among non-Hispanic whites, although blacks have higher rates of mortality. Relative 5-year survival rates are 96%, 68%, and 17% for cancers diagnosed at local, regional, and distant stages, respectively [1].

Endometrial cancers are stratified histologically by responsiveness to estrogen, with the estrogen-dependent type I being most common (90% of cases) and the nonestrogen-dependent type II being less common (10% of cases) [143,144]. Type I tumors are typically low grade and have a good prognosis, whereas type II tumors are more aggressive, tend to be diagnosed in later stages, and are associated with poor prognosis [145]. High cumulative exposure to estrogen is the major risk factor for type I endometrial cancer, whereas exposure to progesterone is protective [1]. Hence, early menarche, late menopause, and nulliparity are risk factors for type I endometrial cancer, just as they are for breast and ovarian cancers [1]. However, unlike these cancers, risk factors for type I endometrial cancer also include tamoxifen use and factors associated with unopposed estrogens [146]. Risk factors for type II endometrial cancer are less well understood because most of the epidemiology study cohorts currently have too few cases to evaluate these tumors separately.

A Nutritional Factors and Endometrial Cancer Risk

1 Height, Weight, and Body Fat Distribution

The link between obesity and endometrial cancer risk is well-established. A 2007 meta-analysis of 23 cohort studies conducted by the WCRF/AICR found that endometrial cancer risk increased by 52% per 5 kg/m^2 increase in BMI [13]. Research suggests that biomarkers associated with adiposity, such as higher circulating levels of leptin and IGFs and lower levels of adiponectin, may also be independently linked with increased endometrial cancer risk [142]. Data from two large cohort studies (EPIC and the AARP Diet and Health Study) suggest that waist circumferences greater than 88 cm and increased waist-to-hip ratio are associated with increased endometrial cancer risk, independent of body weight status [147,148]. Both studies also found that compared to women who are weight stable, those who gain at least 20 kg during adulthood have an increased risk that is roughly two or three times higher [147,148]. Given the strong associations between endometrial cancer and obesity and other markers of adiposity, it is somewhat surprising that intentional weight loss of $\geq$20 kg was found to be associated with a nonsignificant risk reduction of only 4% among women participating in the IWHS [46]. Although a BMI $\geq$30 was associated with increased risk of type II endometrial cancer (RR = 2.87; 95% CI, 1.59−5.16) in the CPS-II Nutrition Cohort [149], women with the nonestrogen-dependent type II endometrial cancer are less likely to be obese (OR = 0.45; 95% CI, 0.29−0.70) compared to women with type I endometrial cancer [150].

In the Netherlands Cohort Study (NCS; 226 cases within a cohort of 62,573), an increased risk of endometrial cancer (RR = 2.57; 95% CI, 1.32−4.99) was found for women who were at least 175 cm in height compared to those measuring 160 cm or less [151]. This association is similar and of the same magnitude as found between height and breast cancer, and it points to the potential role of growth factors and early nutritional status in the etiology of this disease.

2 Dietary Composition

Although obesity is clearly a strong risk factor for endometrial cancer, there is significantly less research available related to specific dietary components and risk of endometrial cancer. Several large cohort studies [152−154] found no associations with carbohydrate intake and either an absence or very weak association between GI or GL and endometrial cancer risk. However, these factors may increase risk for obese versus normal-weight women [152,153] or for nondiabetic women versus women with diabetes [154]. Similarly, two cohort studies found no association between dietary fat intake and endometrial cancer risk [155,156]. A meta-analysis conducted by a workgroup of the International Agency for Research on Cancer (World Health Organization) found no association between fruit and vegetable consumption and endometrial cancer risk (OR = 1.03; 95% CI, 0.9−1.17) [157]. Similarly, few associations for individual plant food constituents, such as lycopene or fiber, have been reported; however, a study by Horn-Ross and colleagues [158] suggests that isoflavones may be protective (RR = 0.59; 95% CI, 0.37−0.93 for highest vs lowest quartiles). In this study, obesity was

found to interact with phytoestrogen intake, suggesting a sevenfold increase in risk among obese women with the lowest intakes of phytoestrogens.

Whereas Jain et al. [155] found no association between folic acid intake and endometrial cancer risk in general among the Canadian National Breast Screening Study cohort, a report from the IWHS cohort [159] stratified endometrial cancer cases by histologic stage. No associations were observed for type I endometrial cancer; however, for type II endometrial cancer, dietary or supplemental folate, methionine, and vitamins B_2, B_6, and B_{12} were associated with increased risk [159].

Finally, although reports from case–control studies suggest a protective benefit of various foods, such as coffee [160] and fatty fish [161], or nutrients, such as vitamin C [162], calcium [160,163], and vitamin D [163], further research is necessary to corroborate these findings. Cohort studies have reported no association with endometrial cancer risk for vitamin A [155], serum retinol [164], vitamin C [155], or vitamin E [155].

Alcohol use has been shown to be associated with elevated circulating estrogen levels and reduced progesterone; however, a meta-analysis found no association between alcohol intake and endometrial cancer risk among cohort or case–control studies [13]. Small sample sizes, limited range of alcohol intake, and confounding factors (e.g., possible interaction with exogenous estrogens or factors such as age) may have limited the ability to detect associations [165,166]. In addition, no study to date has evaluated potential differences in risk by the two histologic subtypes. Thus, more research is needed to clarify the role of alcohol in the etiology of this cancer.

B Nutritional Factors and Endometrial Cancer Recurrence, Progression, and Survival

Comparatively little is known regarding nutritional issues after the diagnosis of endometrial cancer, although research on this topic is increasing. Data regarding the effect of obesity on recurrence, disease-free survival, and overall survival are conflicting. For example, obesity was found to be unassociated with recurrence in one study [167] but inversely associated with recurrence in two others [168,169]. Furthermore, in two studies conducted in the United States, obesity was associated with increased overall mortality [167,170], whereas in a smaller study conducted in Poland, it was found to be protective [171]. Thus, the only consensus regarding obesity and survivorship of endometrial cancer relates to comorbid conditions, in which obesity was found to be associated with the increased prevalence of diabetes, hypertension, and pulmonary disease [168,170] and was also found to be consistently associated with poorer quality of life [172–174].

To date, no studies have investigated other nutritional issues beyond weight status in endometrial cancer survivors. However, the Survivors of Uterine Cancer Empowered by Exercise and Health Diet (SUCCEED) intervention study is currently underway and will evaluate the effect of a dietary intervention (2 cups fruit, 2.5 cups vegetables, three servings of low-fat dairy foods, and three servings whole grain foods per day) on quality of life and treatment outcomes [175].

IV OVARIAN CANCER

Ovarian cancer is the most lethal of the gynecologic cancers, being the fifth most common cause of cancer death among U.S. women despite having a relatively low incidence rate [1]. Women diagnosed with ovarian cancer typically present at an advanced stage of the disease because the early stages are asymptomatic. The established risk factors for ovarian cancer are older age (>50 years), low parity, never having used oral contraceptives, and having a family history of breast or ovarian cancer [176,177]. Although the etiology is still a focus of intense investigation, one theory is that increased ovulation or hormonal stimulation of ovarian epithelial cells plays a role in the development of ovarian cancer [178,179]. Prevention strategies for women at high risk include oral contraceptive use, tubal ligation, and prophylactic oophorectomy [180]. Relatively few studies on the relationship between nutritional factors and risk for ovarian cancer have been reported, and there is a dearth of research that examines how these factors may influence progression or survival after the diagnosis of ovarian cancer.

A Nutritional Factors and Ovarian Cancer Risk

1 Height, Weight, and Body Fat Distribution

Anthropometric factors have been studied extensively with regard to ovarian cancer risk but remain inconclusive. A pooled analysis of 12 prospective cohort studies (2036 ovarian cancer cases among 531,583 women) found that adult attained height of ≥170 cm (67 in.) was associated with an increased risk of ovarian cancer (RR = 1.38; 95% CI, 1.16–1.65) [181]. BMI was not associated with ovarian cancer risk in postmenopausal women, but premenopausal women with a BMI ≥30 kg/m^2 were at increased risk of ovarian cancer (RR = 1.72; 95% CI, 1.02–2.89) compared to women with a BMI between 18.5 and 23 kg/m^2 [181]. In contrast, the EPIC study (611 ovarian cancer cases among 226,798 women) found that BMI ≥30 kg/m^2 was associated with increased risk for all women combined

(HR = 1.3; 95% CI, 1.05−1.68); however, when stratified by menopausal status, the observed increase in risk was only statistically significant among postmenopausal women [182]. Attained adult height, weight change during adulthood, and measures of body fat distribution (waist circumference, waist:hip ratio, or hip circumference) were not found to be associated with risk of ovarian cancer [182]. The findings suggest that the relationship between obesity and ovarian cancer risk may differ from the relationships observed between obesity and risk for either postmenopausal breast cancer or endometrial cancer.

2 Dietary Composition

Similar to the evidence for breast cancer, international comparisons of the incidence of ovarian cancer indicate a strong inverse relationship with per capita dietary fat consumption [183], and the hypothesis that dietary fat intake is associated with increased circulating estrogen concentrations (discussed previously) has stimulated several observational studies of the relationship between dietary fat intake and risk for ovarian cancer. However, a pooled analysis of 12 prospective cohort studies (2132 cases of epithelial ovarian cancer among 523,217 women) found no association between total fat, mono- or polyunsaturated fats, *trans*-unsaturated fat, cholesterol, animal or vegetable fat, or egg intake, and ovarian cancer risk [184]. Similarly, data from the NCS (340 ovarian cancer cases among 62,573 postmenopausal women) found no association between total fat, saturated fat, mono- or polyunsaturated fat, or fat source (animal, plant, dairy, meat, or fish), and ovarian cancer risk; however, *trans*-unsaturated fatty acid intake was found to be associated with increased risk of ovarian cancer (RR = 1.51; 95% CI, 1.04−2.20 for highest vs lowest quintile) [185].

Dietary carbohydrate has been less well studied with respect to ovarian cancer risk. A report from the Canadian National Breast Screening Study (264 ovarian cancer cases among 49,613 women) found that GL was positively associated with ovarian cancer risk (HR = 1.72; 95% CI, 1.13−2.62); however, dietary carbohydrate, sugar intake, and GI were not associated with ovarian cancer risk [186]. A study from the Swedish Women's Lifestyle and Health Cohort (163 ovarian cancer cases among 47,140 women) found no association between dietary fiber and ovarian cancer risk [187].

A report from the NHS found that women who consumed at least 2.5 servings of vegetables and fruit as adolescents had a 46% reduction in risk for ovarian cancer [188], although adult vegetable and fruit intake was unrelated to ovarian cancer risk. A subsequent evaluation of specific dietary flavonoids in this same cohort found no association for total flavonoid intake; however, a statistically significant decrease in ovarian cancer risk was observed for the highest versus lowest quintile of kaempferol intake (RR = 0.75; 95% CI, 0.49−0.91) [189]. These findings were consistent with inverse associations with ovarian cancer observed for nonherbal tea and broccoli (the primary contributors of kaempferol intake in the cohort). Among participants in the California Teachers Study (280 ovarian cancer cases among 97,275 women), women consuming >3 mg/day of soy isoflavones had a significantly decreased risk of ovarian cancer (RR = 0.56; 95% CI, 0.33−0.96) compared to those consuming <1 mg total isoflavones/day [190]. Dietary carotenoids were not associated with ovarian cancer risk in two large prospective cohort studies [188,191].

One small prospective cohort study (35 cases and 67 controls) examined associations between serum concentrations of micronutrients and risk for ovarian cancer [192] using sera collected prior to diagnosis. It found no relationship between risk and serum retinol, β-carotene, lycopene, and lipid-adjusted α-tocopherol and γ-tocopherol concentrations, but serum selenium concentration was inversely associated with risk of ovarian cancer among cases diagnosed 4 or more years after blood collections (p-trend = 0.02) [192]. In contrast to these findings linking serum selenium to risk, another prospective study did not find any association between toenail selenium level and ovarian cancer risk [193]. A large pooled analysis of data from seven prospective cohort studies found no association between serum 25-hydroxyvitamin D levels and ovarian cancer risk [194].

A meta-analysis of two cohort and seven case−control studies found no association between tea intake and ovarian cancer risk (RR = 0.84; 95% CI, 0.66−1.07) [195]; however, important limitations, such as lack of detailed assessment of type of tea consumed, frequency of tea consumption, and duration of tea intake, limit the ability to make a firm conclusion [196]. A meta-analysis of five cohort studies found a statistically significant decrease in risk of ovarian cancer with higher versus lower tea consumption (RR = 0.71; 95% CI, 0.55−0.93), and it also found a marginally increased risk for higher versus lower coffee consumption (RR = 1.32; 95% CI, 0.99−1.77) among four cohort studies [197]. A subsequent report from the IWHS (266 ovarian cancer cases among 29,060 postmenopausal women) supported those findings by observing that women who consumed five or more cups per day of caffeinated coffee were at increased risk of ovarian cancer compared to nonusers (HR = 1.81; 95% CI, 1.10−2.95) [198].

B Nutritional Factors and Ovarian Cancer Recurrence, Progression, and Survival

Only a small number of studies have examined nutritional factors related to survival after an ovarian cancer

diagnosis. A meta-analysis found no association between obesity at the time of diagnosis and subsequent prognosis [199]. Similarly, a retrospective review of 792 advanced ovarian cancer patients participating in a randomized clinical trial of cisplatin/paclitaxel versus carboplatin/paclitaxel found no association between prechemotherapy BMI and survival; however, weight gain during treatment was associated with a statistically significant improvement in overall survival (68.2 vs 48.0 months for >5% increase vs >5% decrease, $p = 0.01$) [200]. A study of 198 advanced ovarian cancer patients undergoing primary surgery and adjuvant chemotherapy found that weight change in 6 months after completion of treatment had no effect on progression-free or overall survival [201].

In a prospective cohort study of 609 women with invasive epithelial ovarian cancer, a statistically significant survival advantage was observed for women who reported higher vegetable intake in general (HR = 0.75; 95% CI, 0.57−0.99) and cruciferous vegetables in particular (HR = 0.75; 95% CI, 0.57−0.98) [202]. A longitudinal study of 341 women with ovarian cancer found that women who consumed diets higher in total fruits at baseline had a significantly decreased likelihood of death during the follow-up period (HR = 0.61; 95% CI, 0.38−0.98), whereas women who consumed diets high in meats (HR = 2.28; 95% CI, 1.34−3.89) or milk (HR = 2.15; 95% CI, 1.2−3.84) were at increased likelihood of death [203].

V SUMMARY AND CONCLUSION

Although a considerable amount of research has been devoted to understanding the effects of nutritional factors on the risk for breast cancer, much remains to be learned. More research on the relationships between these factors and risk for endometrial and ovarian cancers is sorely needed, and to date, few studies have examined how these factors may influence overall survival in women who have been diagnosed with hormone-related cancers. At this time, guidelines from the American Cancer Society [204,205] and the WCRF/AICR [13] form the basis of current dietary recommendations, with both groups emphasizing the importance of achieving and maintaining a healthy weight; eating a diet rich in vegetables, fruits, and whole grains; and limiting meat and alcohol consumption.

The risk for morbidity and mortality from causes other than breast, endometrial, and ovarian cancer should also be considered in dietary recommendations for women and men at risk for cancer and for cancer survivors, especially those diagnosed with early-stage cancers [107]. For example, although evidence to support a link between fat intake and breast cancer risk and prognosis is inconsistent, limiting saturated fat intake is an established strategy to reduce risk for cardiovascular disease. Similarly, eating a diet with adequate dietary fiber has been associated with decreased risk of coronary heart disease and may contribute to overall health [206], irrespective of a specific link between fiber and hormone-related cancers. Diets that emphasize vegetables, fruit, whole grains, low-fat dairy foods, and lean meats and poultry have been associated with decreased risk of all-cause mortality [207].

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Chapter 35

Nutrition and Prostate Cancer

Song-Yi Park and Laurence N. Kolonel
University of Hawaii, Honolulu, HI, United States

I INTRODUCTION

In this chapter, we discuss the epidemiologic evidence for associations of dietary factors with prostate cancer risk and the potential for diet to play a role in prostate cancer prevention. We begin with some general background on the disease and its diagnosis, followed by a description of incidence patterns and risk factors for prostate cancer other than diet. The relationship of nutrition to prostate cancer includes foods and dietary constituents that have been associated with increased risk of the disease, as well as those that have been associated with decreased risk. Some findings from animal and in vitro studies, as well as possible mechanisms for the carcinogenic effects, are presented in support of the epidemiologic findings. We conclude with a few comments on genetic studies of prostate cancer.

A Normal Prostate Anatomy and Function

The normal adult prostate gland is a walnut-sized organ that surrounds the urethra and the neck of the bladder. The gland is composed of three distinct zones: peripheral, central, and transition. The peripheral zone is composed of left and right lobes that can be palpated during digital rectal examination. The transition zone is the region that enlarges in benign prostatic hyperplasia (BPH), which is common in older men [1]. The prostate gland is a male secondary sex organ that secretes one fluid component of semen. Prostatic fluid is essential for male fertility.

Normal growth and activity of the prostate gland is under the control of androgenic hormones. Circulating testosterone, primarily produced in the testes, diffuses into the prostate, where it is irreversibly converted by the enzyme steroid 5α-reductase type II to dihydrotestosterone (DHT), a metabolically more active form of the hormone. DHT binds to the androgen receptor, and this complex then translocates to the cell nucleus, where it activates selected genes [2].

B Pathology and Diagnosis of Prostate Cancer

Almost all prostate tumors are classified as adenocarcinomas (i.e., they arise from the glandular epithelial cells) and occur most commonly in the peripheral zone of the gland. Accordingly, they can often be felt by the physician during digital rectal examination. A unique feature of human prostate cancer is the high frequency of small, latent tumors in older men. A clear relationship between these occult tumors and those that become clinically apparent has not been established, although it is commonly assumed that the latter evolve from the former as a consequence of additional genetic mutations.

Generally, prostate cancer in its early stages is asymptomatic. Enlargement of the prostate gland (BPH) commonly begins after the age of 45 years, ultimately leading to urinary tract symptoms (difficult and frequent urination). Many cases of prostate cancer are diagnosed as a result of digital rectal examination performed when a man visits his physician for relief of these symptoms. (Suspicious lesions on examination may be confirmed by transrectal ultrasound, followed by a biopsy of the gland.) Since its approval by the U.S. Food and Drug Administration in 1986, the prostate-specific antigen (PSA) test has come into widespread use. This test is not specific for prostate cancer, however, and gives an abnormal result if there is any increased tissue growth in the gland, such as occurs in BPH. Because of its sensitivity, the PSA test can lead to the diagnosis of very early, microscopic tumors. Although such lesions might never progress to clinical disease, surgical removal carries a risk of major complications (notably incontinence and/or

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impotence), leading to controversy regarding the proper use of PSA as a screening test for early prostate cancer [3,4]. Indeed, in its review in 2011, the U.S. Preventive Services Task Force (USPSTF) concluded that use of PSA as a screening modality has not resulted in any decrease in prostate cancer mortality and that, to the contrary, its use has led to more harm than good [5]. Thus, in 2012, the USPSTF published a statement recommending against PSA-based screening for prostate cancer [6]. Based on national surveys in the United States, the proportion of men aged 50 years and older reporting PSA screening in the past year was 36.9% in 2005, 40.6% in 2008, and 37.8% in 2010, but after the 2012 USPSTF recommendation, declined to 30.8% in 2013 [7].

II DESCRIPTIVE EPIDEMIOLOGY OF PROSTATE CANCER

A Incidence and Mortality Trends

Prostate cancer is a common cancer among men in many Western countries, and it is the leading male incident cancer in the United States, where 180,890 new cases are projected for the year 2016 [8–10]. Incidence trends in the United States show a rather slow increase over most of the past 50 years, with a striking increase between 1989 and 1992, attributable in large measure to the widespread adoption of the PSA screening test, which first became available in the early 1980s [11,12]. After 1992, the incidence declined until approximately 1995, remained stable until 2000, and then declined again after 2000 [13]. Specifically, from 2003 to 2012, rates decreased by 4% per year [10]. Moreover, mortality from prostate cancer is low relative to its incidence. This is because prostate cancer is generally well controlled by treatment (surgery, radiation, and androgen ablation) and occurs at relatively late ages so that even men who are not cured of the disease often die from other causes. Interestingly, a parallel increase in prostate cancer mortality did not occur during the period 1989–92, presumably because most of the additional cases diagnosed would not otherwise have led to fatal outcomes. Prostate cancer mortality rates decreased by 3.5% per year from 2003 to 2012, attributed to improvements in early detection and treatment [10].

B Risk Factors for Prostate Cancer

Few risk factors for prostate cancer have been established. Proposed factors are listed in Table 35.1. Age is the strongest risk factor. Prostate cancer incidence increases more sharply with age than does any other cancer; about 60% of cases in the United States are diagnosed in men 65 years of age or older [9,14].

Race/ethnicity is a second risk factor for prostate cancer. In the United States, the lowest incidence rates (cases/100,000) are seen among Cambodian (29.1) and Laotian (32.3) men, followed by Vietnamese (45.3) and Korean (48.3) men, relatively recent immigrant groups from Asia; the rates are higher among Chinese (66.4), Japanese (99.9), Filipino (101.8), American Indian/Alaska Native (90.5), and Native Hawaiian (101.8) men [10,15]. Caucasian men have very high rates, but by far the highest incidence of this cancer is among African American men [10].

The incidence of prostate cancer varies widely in populations throughout the world (Fig. 35.1). Indeed, of all common malignancies, this cancer shows the widest variation between low- and high-risk countries or populations. High rates are seen in developed, especially Western, countries, including the United States, Canada, areas of Europe, and Australasia. Low rates tend to occur in Asia [9]. The highest reported rates in the world are among African Americans, whereas the lowest reported rates are among men in China and India. Interestingly, Chinese men in more developed areas of Asia (Singapore and Hong Kong) and Chinese men in the United States have much higher incidence rates than men in mainland China (see cross-hatched populations in Fig. 35.1). Furthermore, immigrants from Japan to Brazil and the United States have higher rates than do men in Japan [16]. The incidence of prostate cancer in Japan increased approximately tenfold between 1975 and 2011 [17],

TABLE 35.1 Proposed Risk Factors for Prostate Cancer

Category	Characteristic or Exposure
Demographic	Age, ethnicity, geography
Genetic	Family history (father, brothers), rare high-penetrance genes, more common susceptibility genes
Occupational	Cadmium products, rubber industry, agricultural chemicals
Hormonal	Androgens (testosterone, DHT)
Lifestyle	Sexually transmitted agents, smoking, alcohol, vasectomy, physical activity, diet

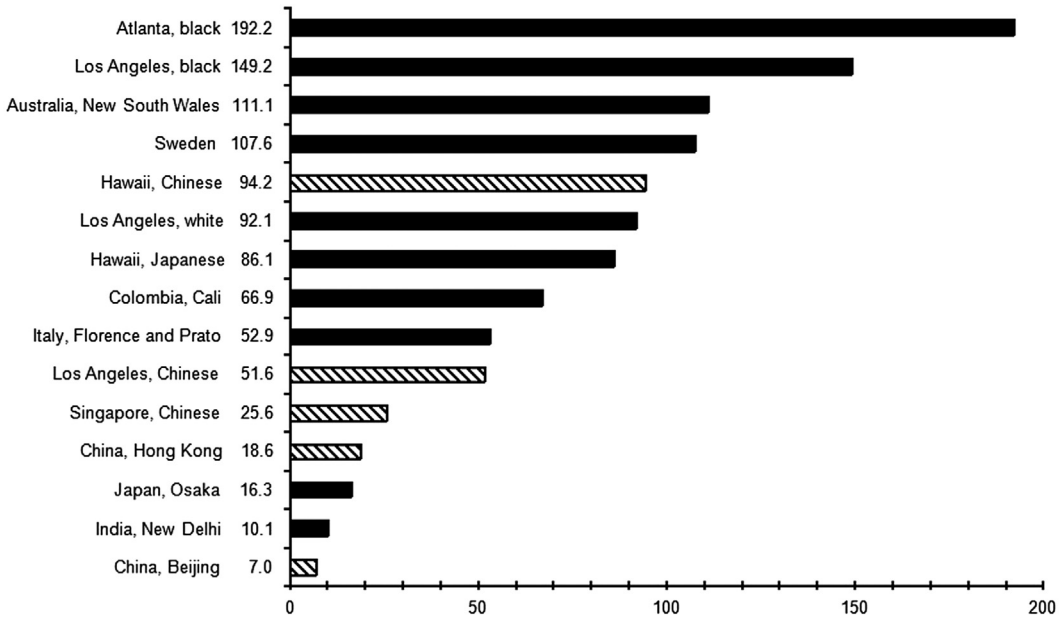

FIGURE 35.1 Prostate cancer incidence per 100,000 in selected populations, 2003–07 (rates age-adjusted to the World Standard Population).

although the actual incidence in Japan is still low compared with that of Japanese men in the United States.

Men with a first-degree male relative who has had prostate cancer are at a two- or threefold increased risk; whether this reflects an inherited predisposition for the disease or a shared environmental exposure has not been confirmed [18,19]. The search for high-penetrance, rare genes for prostate cancer has identified some candidates, although none has yet been confirmed; of even greater interest is the potential role in this disease of low-penetrance, highly prevalent susceptibility genes (discussed later).

Apart from these few established risk factors, the etiology of prostate cancer is unknown. Among the several potential causal agents, apart from diet, that have been proposed are: (1) occupational exposures (rubber industry; manufacture of products containing cadmium, such as paints and batteries; and use of agriculture chemicals), (2) sexually transmitted agents (e.g., gonorrhea, syphilis, and human papillomavirus), (3) smoking, (4) alcohol use, (5) vasectomy, and (6) physical activity [20–30]. However, the evidence is not convincing for any of these exposures.

Although it is suspected that most exogenous factors affecting prostate cancer risk exert their influence by altering endogenous androgen levels [31,32], epidemiologic studies have not clearly established the role of androgens in prostate cancer [33]. Some studies have supported a positive relationship of circulating androgen levels to prostate cancer risk [34–38], but others have not [39–45]. In a pooled analysis of 18 prospective studies, prediagnostic circulating levels of individual androgens were not associated with prostate cancer, whereas the sex hormone-binding globulin level was modestly inversely associated with prostate cancer [46]. Further studies are warranted to understand the role of androgens in tissue, androgen action in the prostate, and the relationship between tissue and blood levels of androgen in the causation of prostate cancer [47].

The most promising area of research, apart from genetics, on the etiology of prostate cancer pertains to diet.

III STUDIES OF DIET IN RELATION TO PROSTATE CANCER

A Origin of the Diet–Prostate Cancer Hypothesis

The descriptive patterns of prostate cancer, especially data showing very different rates of the disease in the same ethnic/racial group living in different geographic settings, as well as changing rates in migrants and their offspring [16], prompted investigators to seek environmental risk factors for this cancer. Diet became an important focus of this research because (1) geographic variations in food and nutrient intakes are known to be large [48] and (2) components of the diet can influence the levels of circulating androgens [49], which, as noted previously, are thought to play a role in prostate cancer risk. Many different dietary factors, including both foods and particular constituents of foods, have been proposed and studied. Some of these appear to increase risk,

TABLE 35.2 Proposed Dietary Risk Factors for Prostate Cancer

Increasing Risk	Decreasing Risk
Foods and Beverages	
Processed and red meat	Vegetables
Milk and dairy products	Fruits
Alcohol	Legumes
	Tea and coffee
Food Components	
Total energy	Vitamin D
Fat	Vitamin E
Calcium	Carotenoids
Zinc	Selenium
Cadmium	Isoflavonoids
Diet-Associated Factors	
Obesity, weight, and height	Physical activity

whereas others are possibly protective. These factors are listed in Table 35.2, and the supporting evidence is discussed in the following sections.

B Dietary Factors that Increase Risk

In 2007, the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) produced an authoritative report, titled *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*, containing recommendations to reduce the risk of cancer [48]. These recommendations were based on comprehensive systematic literature reviews (SLRs) that were evaluated by an international panel of experts, including one of the present authors (LNK) [50]. Following the 2007 report, the WCRF International established the Continuous Update Project (CUP) to update the SLRs on an ongoing basis and to reconsider the recommendations as needed. The CUP published its latest report on the updated evidence for prostate cancer in 2014, covering all major databases up to April 2013, and available online at http://www.wcrf.org/int/research-we-fund/continuous-update-project-findings-reports/prostate-cancer [51]. The 2014 SLR considered randomized controlled trials and cohort studies, but not case−control studies (which had been included in prior reviews) due to their methodological limitations, especially the potential for recall bias which is a major concern for epidemiologic studies of diet and nutrition [52]. Thus, in the following sections, we use the conclusions of the 2014 CUP report as a starting point, and then review the findings from more recent studies, if any, to make an overall assessment of the literature. In its judgments, the CUP expert panel graded the evidence in five categories, as in the 2007 report: convincing, probable, limited—suggestive, limited—no conclusion, and substantial effect on risk unlikely. Recommendations were based only on the highest two categories.

1 Dietary Patterns

Foods are generally consumed in many combinations, and together they may have a greater impact on cancer risk than as individual components [53]. Thus, a dietary pattern approach that reflects the complexity of dietary intake may provide a more comprehensive assessment of the role of diet in cancer causation. Although many different health outcomes have been associated with dietary patterns [54,55], only a few such studies have examined prostate cancer specifically [56−61]. Some patterns, such as those high in consumption of meat, dairy fat, and refined grains (like the so-called Western Diet), were hypothesized to increase prostate cancer risk; while others, such as those high in consumption of fruits, vegetables, and whole grains (like the so-called Mediterranean Diet), were hypothesized to lower prostate cancer risk. The CUP SLR found three studies that examined presumptive high-risk dietary patterns in relation to prostate cancer risk [52]. Two of these examined dietary patterns that had been developed from their respective study datasets (i.e., a posteriori dietary patterns). One of these, a cohort study of U.S. health professionals [56], identified a Western dietary pattern, and the other, an Australian cohort study [57], identified a meat and potatoes pattern but neither pattern was associated with prostate cancer risk. The third study, based on a cohort in Japan [59], found no association of a dietary preference for fatty foods with prostate cancer mortality. Though the evidence at present is limited, the data do not suggest that presumptive high-risk dietary patterns are associated with prostate cancer incidence or mortality.

2 Foods and Beverages

a Processed and Red Meat

The CUP report reached no conclusions regarding processed meat and prostate cancer due to inconsistent evidence [51]. The SLR found 11 cohort studies that met the criteria for a dose-response meta-analysis, as defined in the SLR [52]. The meta-analysis showed no significant association; the summary relative risk estimate per 50 g/day was 1.03 (95% confidence interval, CI: 0.98, 1.08).

A great many epidemiologic studies have investigated associations between red meat consumption and prostate cancer risk. However, the findings are not consistent. The CUP dose−response meta-analysis of eight cohort studies produced nonsignificant summary risk estimates and led to no conclusion by the expert panel. More recent meta-analyses also did not support an association with processed or red meat intake [62,63].

Explaining a possible association with meat intake is not straightforward. Early reports of positive findings were thought to reflect a high exposure to dietary fat, especially saturated fat, because meat and dairy products are the major contributors to fat intake in the Western diet. However, because the findings on dietary fat per se and prostate cancer are equivocal (discussed later), other explanations for an association with meat needed to be considered. There are several possibilities. First, in the American diet, meat is a major source of zinc, which is essential for testosterone synthesis and may have other effects in the prostate (discussed later). Second, diets high in meat and other animal products may be relatively deficient in certain anticarcinogenic constituents found primarily in plant foods. Third, red meat contains high levels of heme iron, which is a source for free radical formation and oxidative damage to tissues [64]. Fourth, nitrates added as preservatives to processed meat may contribute to *N*-nitroso compound production and exposures. These compounds are suspected mutagens and carcinogens [65]. Heme also promotes the formation of *N*-nitroso compounds. Finally, and most intriguing, many meats are cooked at high temperatures, such as by pan frying, grilling, or barbecuing. Cooking meats at high temperatures can result in the formation of heterocyclic amines (HAs), which are potent carcinogens in animals, including the rat prostate [66,67]. Furthermore, when meats are cooked on charcoal grills, rendered fat is pyrolized by the coals, leading to the deposition of polycyclic aromatic hydrocarbons (PAHs), which are also carcinogenic in animals, on the outer surface of the meat [68]. Although an accurate assessment of dietary intake of HAs and PAHs is difficult, two studies reported a positive association of prostate cancer with estimated intakes of very well-done meat and of a particular HA (PhIP) [69,70], especially for advanced tumors [70]. However, other studies that examined intake of well-done meat [71] or estimated HA and PAH intakes from cooked meat [72–76] in relation to risk of prostate cancer as well as a meta-analysis of five prospective studies [63] did not provide clear support for the hypothesis.

b Milk and Dairy Products

The CUP report concluded that there was limited evidence suggesting that dairy products are a cause of prostate cancer. In the SLR, 21 cohort studies were identified and 13 reported a positive association when comparing the highest versus the lowest categories of intake. The dose–response meta-analyses of 15 studies showed a 7% increased risk per 400 g of dairy products per day (the summary relative risk estimate = 1.07, 95% CI: 1.02, 1.12) (Fig. 35.2) [77]. With regard to milk specifically,

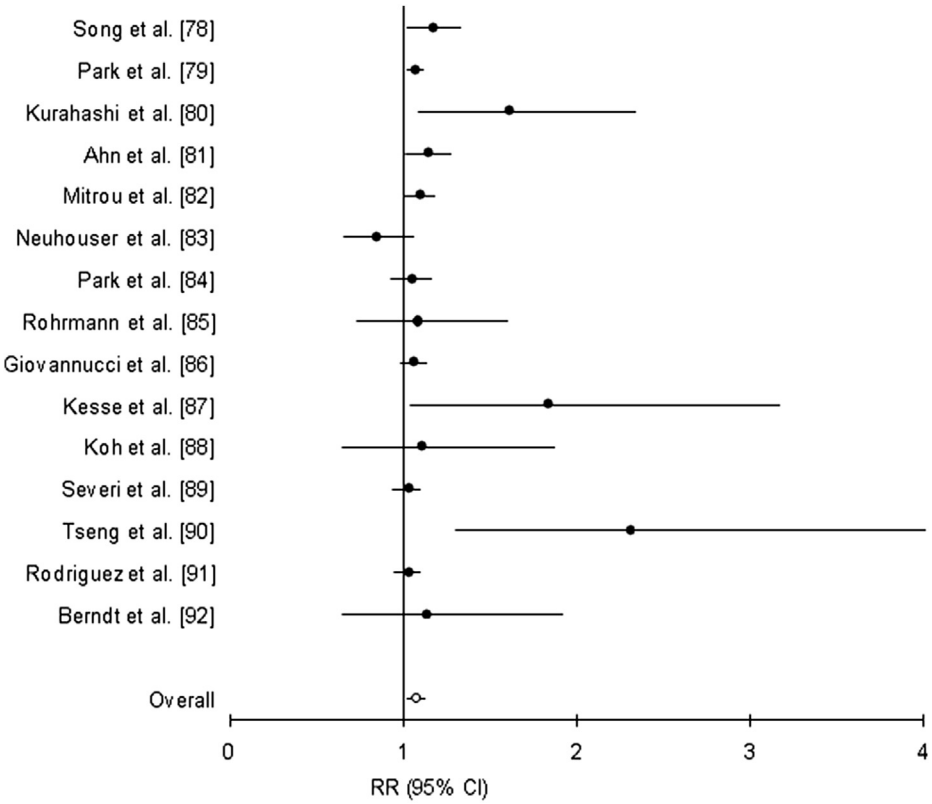

FIGURE 35.2 Dairy product intake and prostate cancer in cohort studies. *RR*, relative risk, per 400 g/day; error bars indicate 95% CIs. *Adopted, with permission of the American Society for Nutrition, from D. Aune, D.A. Navarro Rosenblatt, D.S. Chan, A.R. Vieira, R. Vieira, D.C. Greenwood, L.J. Vatten, T. Norat, Dairy products, calcium, and prostate cancer risk: a systematic review and meta-analysis of cohort studies, Am. J. Clin. Nutr. 101 (1) (2015) 87–117.*

the summary relative risk estimate per 200 g of milk intake per day was 1.03 (95% CI: 1.00, 1.06) for total prostate cancer based on 14 studies and 1.06 (95% CI: 1.00, 1.13) for nonadvanced prostate cancer based on four studies, suggesting a borderline significant association [77].

A possible explanation for the positive associations is an adverse effect on the prostate of the high fat, especially saturated fat, content of dairy products. Another prominent constituent of these foods is calcium, which has also been proposed as a risk factor for prostate cancer (discussed later). Finally, milk consumption may increase blood levels of insulin-like growth factor-I (IGF-I), which has been associated with increased prostate cancer risk in some studies [93].

c Alcoholic Beverages

The CUP meta-analyzed 25 cohort studies, which yielded a summary relative risk estimate of 1.01 (95% CI: 0.99, 1.02) for an increase of one alcoholic drink per day. The expert panel determined that the evidence was too limited for a firm conclusion as to whether alcohol consumption was associated with prostate cancer risk. Since the SLR, two cohort studies found no association between alcohol intake and prostate cancer risk [94,95].

Some general mechanisms by which alcohol might enhance carcinogenesis have been proposed, including the activation of environmental nitrosamines, production of carcinogenic metabolites (acetaldehyde), immune suppression, and secondary nutritional deficiencies [96–98].

3 Nutrients and Other Food Constituents

a Energy

The findings from studies that have examined total energy intake in relation to prostate cancer are very inconsistent. No conclusion was made about energy intake in the CUP report. The meta-analysis in the SLR of eight cohort studies showed no significant dose–response association. No study has reported the relationship between energy intake and prostate cancer since the SLR.

An experimental study in rodents (rats and mice) found that energy restriction reduced prostate tumor growth, possibly by inhibiting tumor angiogenesis [99].

b Fat

Dietary fat has been the most studied nutrient with regard to effects on prostate cancer risk. Detailed reviews on this topic have been published [100–103]. The SLR in the CUP report yielded no significant association for total or saturated fat consumption, either as grams or as percentage energy, in a meta-analysis of nine cohort studies. However, because the evidence was limited, the expert panel reached no conclusion about total and subtypes of dietary fat in relation to prostate cancer. A recent meta-analysis also did not support a dose–response association between total or saturated fat intake and prostate cancer risk [103].

Some epidemiologic studies examined dietary intakes of monounsaturated and polyunsaturated fat as well. The CUP SLR included seven cohort studies in the meta-analysis for monounsaturated fatty acids, with a summary relative risk estimate of 1.00 (95% CI: 0.99, 1.01) per 10 g/day. The meta-analyses for dietary polyunsaturated fatty acids were based on seven cohort studies and did not show any association.

Several studies have also examined specific fatty acids (including several omega-3 and omega-6 polyunsaturated fatty acids), based either on dietary intake data or on biochemical measurements in blood or adipose tissue [100,104]. A few studies suggested that long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were inversely associated with prostate cancer [105–108], although other studies did not reproduce this finding [109–113]. On the contrary, two nested case–control studies within cohorts reported an increased risk of prostate cancer with high blood concentration of long-chain omega-3 fatty acids [114,115]. Additional support for these inverse findings was provided by studies of fatty fish intake (a good source of long-chain polyunsaturated fatty acids) that also showed inverse associations with prostate cancer risk [116–119]. However, the CUP meta-analysis found no significant association for EPA and DHA, nor for fish consumption overall. The effect of α-linolenic acid (ALA) intake was examined in several studies, with some showing a positive association [106,109,120] and some no association [110–112,121]. A meta-analysis involving eight prospective studies that measured either ALA intake or blood/tissue concentrations supported the positive association with prostate cancer [122]. However, the CUP meta-analysis showed no significant association with ALA based on dietary intake from five cohort studies and biomarker from six cohort studies. Finally, two studies reported that prediagnostic blood concentrations of *trans*-fatty acids were associated with increased risk of prostate cancer [123,124], while another two studies found no association [125,126]. Overall, these reports are not very consistent, and no firm conclusion regarding the role of specific fatty acids on the risk of prostate cancer can be reached on the basis of current data.

Some animal experiments have tested the fat–prostate cancer hypothesis. For example, a high-fat diet increased prostate cancer incidence and shortened the latency period in Lobund–Wistar rats treated with exogenous testosterone to induce the tumors [127]. Conversely, prostate tumor growth rate was reduced by a fat-free diet in Dunning rats [128] or by lowering dietary fat intake in

athymic nude mice injected with LNCaP cells (a human prostate cancer cell line) [129,130]. With regard to specific types of fat, fish oils containing high levels of long-chain omega-3 fatty acids, such as EPA and DHA, generally suppressed prostate tumor growth in rodents, whereas omega-6 polyunsaturated fatty acids, such as linoleic and linolenic acids, promoted tumor growth [119,131,132]. However, because most animal studies have been conducted in rodents, whose prostate glands differ anatomically from that of the human, extrapolation of these findings to humans is particularly tenuous.

A number of plausible mechanisms by which dietary fat could increase cancer risk have been proposed. These include the formation of lipid radicals and hydroperoxides that can produce DNA damage, increased circulating androgen levels, decreased gap-junctional communication between cells, altered activity of signal transduction molecules, effects on eicosanoid metabolism, and decreased immune responsiveness [100].

c Calcium

As noted previously, a number of studies that have examined the relationship of dairy product consumption to prostate cancer risk found a positive association. Dairy products could be a marker of exposure to calcium, although this food group is also a major source of saturated or animal fat in the Western diet. The CUP report concluded that for diets high in calcium, the evidence is "limited-suggestive" for an increased risk of prostate cancer. In the SLR, a total of 16 studies were identified and 13 reported a positive association when comparing the highest versus the lowest categories of dietary calcium intake. A dose–response meta-analysis of 15 cohort studies yielded a summary relative risk estimate of 1.05 (95% CI: 1.02, 1.09) per 400 mg per day for total prostate cancer, 1.07 (95% CI: 1.03, 1.12) for nonadvanced, and 1.02 (95% CI: 0.93, 1.12) for advanced tumors [77]. Since that SLR, one cohort study reported a positive association between dietary calcium and prostate cancer risk, but only with long latency periods [133].

A mechanism for an adverse effect of calcium on prostate carcinogenesis has been proposed based on the observation that a high intake of calcium decreases the circulating levels of $1,25(OH)_2$ vitamin D, which may inhibit cell proliferation and promote differentiation in prostatic tissue [134,135]. The role of vitamin D in prostatic carcinogenesis is discussed later.

d Zinc and Cadmium

The trace elements zinc and cadmium are considered together because they act as antagonists in biological systems. Due to limited evidence, the CUP report categorized zinc in the diet or supplements as "limited-no conclusion."

The frequent association of prostate cancer risk with high intake of red meat (discussed previously) could also be explained by a higher intake of zinc, rather than animal fat, because meat, especially red meat, is an important source of zinc in the American diet (other sources are shellfish, whole grain cereals, nuts, and legumes) [136]. Reports based on zinc levels in blood or prostatic tissue of patients with cancer and controls have not been consistent [137–139], but such studies are unreliable because the levels of zinc measured after diagnosis in the cases may reflect physiologic changes in the prostate as a result of the cancer ("reverse causation"). One study measured prediagnostic toenail zinc in prostate cancer patients and in controls; no association was found [140].

As a major constituent of prostatic fluid [139,141,142], zinc is essential for normal prostate function. Zinc is also essential for normal testicular function, and high levels of zinc have been proposed to increase the production of testosterone, leading to enhanced tissue growth in the prostate. Blood levels of zinc have been positively correlated with testosterone and DHT levels in men [143,144]. Furthermore, in the rat prostate, zinc has been shown to increase 5α-reductase activity [145] and to potentiate androgen receptor binding [146]. Thus, one might speculate that higher intake of zinc could partially offset the normal decline in testosterone levels with age [147–149], thereby contributing to prostate cancer risk. However, an epidemiologic study that analyzed prediagnostic zinc levels in serum in relation to prostate cancer risk reported no clear association [150]. Thus, evidence to support a role of zinc in prostate cancer is still limited, and no firm conclusion can be reached.

Epidemiologic evidence for cadmium as a risk factor for prostate cancer is also limited [151]. The CUP SLR found only three cohort studies that assessed cadmium exposure in relation to prostate cancer risk, and no meta-analysis was conducted. A meta-analysis of one case–control and two cohort studies reported an increased risk with dietary cadmium intake [152]. Since that SLR and the meta-analysis, a Danish cohort study found no association between dietary cadmium and prostate cancer risk [153]. With so few studies and a lack of data from cohorts in particular, no firm conclusion can be reached regarding the association of cadmium exposure with prostate cancer risk.

Cadmium is a competitive inhibitor of zinc in enzyme systems and accumulates in the body throughout life because no mechanism exists for excreting it. Thus, the hypothesis that cadmium may be carcinogenic for the prostate has biologic plausibility. This hypothesis is further supported by studies showing that cadmium is carcinogenic in animals, and that the effect can be blocked by simultaneous injection of zinc [154,155].

4 Diet-Associated Risk Factors

a Obesity, Weight, and Height

Prostate cancer is sometimes considered a male counterpart to breast cancer in women, for which there is clear evidence of a positive association with obesity, especially in postmenopausal cases. However, evidence for a similar association of adult obesity with prostate cancer is much less clear. The CUP concluded that greater body fatness (marked by body mass index, waist circumference, and waist-to-hip ratio) is a "probable cause" of advanced prostate cancer [51]. Of 24 studies reporting on body mass index and advanced prostate cancer risk, 13 reported positive association when compared the highest versus the lowest categories. The dose−response meta-analyses in the SLR yielded a statistically significant risk for advanced, but not for total prostate cancer; summary estimates per 5 kg/m^2 were 1.08 (95% CI: 1.04, 1.12) for advanced prostate cancer based on 23 studies and 1.00 (95% CI: 0.97, 1.03) for total prostate cancer based on 39 studies. Since the CUP, a Danish cohort study reported an increased risk of advanced prostate cancer risk with body mass index [156], while a Swedish cohort study found no association [157]. In a report from a cohort consortium in Asia, body mass index was not associated with prostate cancer mortality [158], while a study in men with prostate cancer found that high body mass index at time of diagnosis was associated with increased overall and prostate cancer-specific mortality [159]. A meta-analysis of 17 cohort studies found that obesity measured by body mass index was a significant risk factor for aggressive prostate cancer and prostate cancer-specific mortality [160]. Some studies examined obesity in early adulthood (age 18−21) or at birth, but the meta-analyses of these studies in the CUP SLR showed no significant overall association. Since then, a cohort study reported an increased risk of prostate cancer with childhood body mass index and height [161,162]. In the CUP SLR, a meta-analysis of 14 cohort studies did not show a significant association of weight with total or advanced prostate cancer risk. However, waist circumference and waist-to-hip ratio were related to an increased risk of advanced cancer; the summary relative risk estimate was 1.12 (95% CI: 1.04, 1.21) per 10 cm and 1.15 (95% CI: 1.03, 1.28) per 0.1 units, respectively.

The CUP concluded that developmental factors leading to greater linear growth (marked by adult attained height) are a "probable cause" of prostate cancer. Of 42 studies identified in the CUP, 25 studies reported on total prostate cancer incidence and 22 reported a positive association, when comparing the highest versus lowest categories of adult attained height. In the SLR, the dose−response meta-analysis yielded a significant association between height and prostate cancer risk; summary risk estimates were 1.04 (95% CI: 1.03, 1.05) per 5 cm based on 34 cohort studies. When stratified by prostate cancer outcome, the meta-analysis showed statistically significant associations; the summary relative risk estimate per 5 cm was 1.03 (95% CI: 1.01, 1.05) for nonadvanced, 1.04 (95% CI: 1.02, 1.06) for advanced, and 1.04 (95% CI: 1.01, 1.06) for fatal prostate cancer. Recent cohort studies confirmed that tallness was associated with an elevated risk of total [156,163,164] and advanced prostate cancer [156], particularly fatal disease [165], although another cohort found no association [95].

The basis for an association between obesity and prostate cancer could involve endocrine factors because adult obesity in men has been associated with decreased circulating levels of testosterone and increased levels of estrogen [166,167]. However, this mechanism would suggest an inverse rather than a direct association between obesity and this cancer. Other possible mechanisms entail pathways that involve insulin, leptin, adipokines, IGF-I, and chronic inflammation [168]. For example, leptin is a hormone produced by adipocytes. Obese men have more and larger adipocytes, and they have been shown to have higher serum levels of leptin [169]. In in vitro studies, leptin promoted proliferation of androgen-independent prostate cancer [170].

5 Dietary Factors that Decrease Risk

a Dietary Patterns

Four cohort studies that examined presumptive risk-reducing dietary patterns in relation to prostate cancer risk were included in the CUP SLR. Two of these studies were based on predefined dietary patterns. A cohort study in the United States found a decreased risk of prostate cancer associated with a higher score on the Healthy Eating Index (HEI) and the alternate HEI, but only for cancers detected through PSA screening [171]. A cohort study in Korea, on the other hand, found that a dietary preference for vegetables rather than meat was not related to prostate cancer risk [58]. Two other cohort studies investigated data-derived (i.e., a posteriori) healthful eating patterns (generally characterized by high intakes of fruits, vegetables, and/or whole grains), and neither study found a significant association with prostate cancer risk [56,57]. Because the patterns investigated varied across studies, no meta-analysis was possible in the CUP SLR [52]. Since the CUP SLR, two additional cohorts have reported their findings. A cohort study in Sweden reported no association of prostate cancer incidence with a modified Mediterranean diet score, but an inverse association of prostate cancer incidence with a higher "low carbohydrate-high protein" score [60]. A cohort study of health professionals in the United States found no association between the traditional Mediterranean Diet and

advanced prostate cancer risk [61]. Thus, similar to the findings for presumptive high-risk dietary patterns, the data for presumptive low-risk dietary patterns do not suggest any association with prostate cancer incidence.

6 Foods and Beverages

a Vegetables

Intake of vegetables has been inversely associated with cancer risk at many sites. This has led to strong recommendations to consume significant quantities of these foods as part of a healthful diet. However, the evidence for a beneficial effect of vegetables on prostate cancer risk is not overwhelming. The CUP report placed vegetables in the "limited-no conclusion" category because of limited evidence. The meta-analyses for all vegetables in the SLR yielded a summary estimate of 0.99 (95% CI: 0.98, 1.00) per 100 g/day based on 13 cohort studies. When restricted to green leafy, yellow-orange, or cruciferous vegetables, summary estimates were not statistically significant. Since that SLR, reports on three prospective cohort studies also observed no relation [172–174]. Thus, even with the additional few studies since the CUP report, there is little evidence for an effect of vegetable consumption overall on prostate cancer risk. The findings for legumes, a vegetable subgroup, are considered separately (discussed later), and the findings for tomatoes are included in the later discussion of carotenoids.

Because vegetables contain numerous compounds that can act through a variety of mechanisms to inhibit carcinogenesis [175,176], an inverse association between vegetables and prostate cancer is plausible. Some of these mechanisms are discussed later with respect to specific food constituents.

b Fruits

The CUP report placed fruit consumption and prostate cancer risk in the "limited-no conclusion" category. The meta-analysis in the SLR yielded no significant association: the summary relative risk estimate was 1.00 (95% CI: 0.99, 1.01) per 100 g/day based on 16 cohort studies. Since that SLR, two cohort studies reported no association [172,173].

Fruits contain many of the same compounds with anticarcinogenic properties that are found in vegetables, such as various carotenoids and vitamin C [177]. Because most of the findings for this food group have been null, it does appear that fruit intake has no particular benefit with regard to the risk of prostate cancer.

c Legumes, Including Soy Products

Prostate cancer rates have traditionally been low in populations, such as those of Japan and China, in which the intake of soy products is relatively high. The CUP report concluded that the evidence was too limited to draw a firm conclusion regarding a role of legumes in increasing or decreasing the risk of prostate cancer. In the SLR, a dose–response meta-analysis was not conduced for legume and soy product intake due to the limited number of eligible cohort studies. Although previous meta-analyses supported an overall inverse association between soy foods consumption and prostate cancer risk [178–180], no recent studies have reported protective effects of soy foods against prostate cancer.

In the past, legumes were of interest in nutritional epidemiology primarily because of their important contribution to fiber intake. However, these foods also contain phytoestrogens, plant constituents that have mild estrogenic properties. Because estrogens are associated with lower risk of prostate cancer and are used in prostate cancer therapy, there is a good rationale for the hypothesis that phytoestrogen intake can protect against prostate cancer. Soybeans and many products made from soy, such as tofu, are rich in a class of phytoestrogens known as isoflavones (other classes of phytoestrogens include the coumestans and lignans). The main isoflavones found in soy include genistein, daidzein, and glycetein [181]. Several epidemiologic studies assessed the intake of dietary phytoestrogens, particularly isoflavones, and found inverse associations with prostate cancer risk [182–187]. Indeed, a meta-analysis suggested that higher phytoestrogen consumption (two cohort and nine case–control studies) and serum concentration (six case–control studies nested within cohorts and two population-based case–control studies) were related to a reduction in prostate cancer risk [188]. Furthermore, an analysis of urinary isoflavone excretion within a large cohort study showed inverse associations, suggesting that high intake of isoflavones may be protective against prostate cancer risk [189]. However, another nested case–control study did not confirm this result [190], and a subsequent large nested case–control study reported no association of prostate cancer with prediagnostic plasma genistein levels [191]. Thus, the evidence in support of a protective effect of legumes and phytoestrogens on prostate cancer risk remains limited and inconsistent.

The mechanism for a benefit of soy products on prostate carcinogenesis could entail the estrogenic effects of isoflavones, although other actions of these compounds, such as inhibition of protein tyrosine phosphorylation, induction of apoptosis, and suppression of angiogenesis, have also been proposed [192]. Laboratory data, based on human tissue as well as animal models, offer support for the hypothesis that soy products may protect against prostate cancer [193–196].

Although soy products and isoflavones are of particular interest, legumes contain other bioactive microconstituents, including saponins, protease inhibitors, inositol

hexaphosphate, γ-tocopherol, and phytosterols. Mechanisms by which each of these compounds can inhibit carcinogenesis have been proposed [192,193,197].

d Tea and Coffee

Due to limited evidence, the CUP report placed tea consumption in the "limited-no conclusion" category. The meta-analyses in the SLR found no support for an association based on five cohort studies. Since that SLR, a cohort study in the United States showed no relationship between daily tea consumption and prostate cancer risk [198], while a Netherlands cohort study found a decreased risk of advanced prostate cancer with black tea consumption [199]. A more recent meta-analysis, including seven prospective studies, did not show a protective effect of tea consumption on prostate cancer risk [200]. Thus, the evidence remains insufficient to draw firm conclusions regarding the effect of tea consumption on this cancer.

Tea contains polyphenols that are potentially anticarcinogenic because of their antioxidant properties, effects on signal transduction pathways, inhibition of cell proliferation, and other actions in the body [201,202].

The consumption of coffee has been examined in relation to prostate cancer risk, since it contains various components with potential anticancer properties. The CUP determined that the evidence for coffee and prostate cancer was "limited-no conclusion." The dose−response meta-analysis in the SLR showed a summary relative risk estimate of 0.99 (95% CI: 0.98, 1.01) per 1 cup/day for total prostate cancer incidence (based on eight cohort studies), and 0.97 (95% CI: 0.93, 1.00) for prostate cancer mortality (based on four studies). Since the CUP, two cohort studies observed no significant association [198,203], while another found a decreased risk [204]. Recently, several meta-analyses have been conducted to investigate the association between coffee and prostate cancer risk. Although one meta-analysis did not support [205], others suggest an inverse association between coffee consumption and prostate cancer [206−209], especially fatal tumors [207,210].

Potential mechanisms for anticancer effects of coffee phytochemicals include inhibition of oxidative stress and oxidative damage, regulation of DNA repair, phase II enzyme activity, apoptosis, inflammation, as well as having antiproliferative, antiangiogenetic, and antimetastatic effects [211].

7 Nutrients and Other Food Constituents

a Vitamin D

Evidence for a protective effect of vitamin D against prostate cancer is not convincing, and, though several studies examined this nutrient, the CUP report determined that the evidence was "limited-no conclusion" possible. However, because circulating vitamin D levels are substantially determined by the conversion of 7-dehydrocholesterol in the skin in response to solar UVB radiation, studies based on dietary vitamin D intake alone may be misleading [212,213].

Several cohort studies have examined the relationship of prediagnostic blood levels of 25-hydroxyvitamin D (25(OH)D), the most abundant circulating form of the vitamin, to subsequent development of prostate cancer. A dose−response meta-analysis in the CUP SLR showed no significant association: the summary risk estimate was 1.04 (95% CI: 1.00, 1.07) per 30 nmol/L based on 17 prospective studies. Since that SLR, a prospective study reported a decreased risk of prostate cancer with higher 25(OH)D concentration in blood [214]. On the contrary, a cohort study reported that lower levels of 25(OH)D in blood may reduce prostate cancer risk in older men [215]. Another study found that both low and high 25(OH)D concentrations were associated with increased risk of prostate cancer, and more strongly for high-grade disease [216]. A consortium of three European cohorts found that lower 25(OH)D concentrations were not significantly associated with increased incidence of prostate cancer [217]. Another consortium of six cohorts found no association between circulating 25(OH)D and fatal prostate cancer risk [218]. Thus, there is little epidemiologic data to support the hypothesis of a protective effect of vitamin D against prostate cancer, with some evidence even suggesting an adverse effect at high circulating levels of 25(OH)D.

The hormonal form of vitamin D, $1,25(OH)_2D$, reduces cell proliferation in the prostate (and other tissues) and enhances cell differentiation, both of which would be expected to lower the risk of cancer [134,219].

b Vitamin E

The CUP report concluded that evidence was too limited to draw a conclusion that vitamin E intake decreases or increases the risk of prostate cancer. In the CUP meta-analyses, the summary risk estimate was 1.01 (95% CI: 0.96, 1.06) per 10 mg/day for dietary vitamin E based on five cohort studies.

Several cohort studies have reported findings for vitamin E and prostate cancer based on prediagnostic blood levels. The CUP concluded that the evidence for an association between low plasma γ-tocopherol levels and increased prostate cancer risk was "limited-suggestive." Of 11 studies identified in the SLR, 8 reported an inverse association when comparing the highest versus the lowest γ-tocopherol concentration. A dose−response meta-analysis in the SLR yielded summary estimates of 0.99 (95% CI: 0.98, 1.00) per 1 mg/L of γ-tocopherol in blood based on nine cohort studies (Fig. 35.3) and of 0.97 (95% CI: 0.91, 1.04) per 1 mg/L of γ-tocopherol in blood based on seven cohort studies.

The CUP report concluded that the evidence for vitamin E supplements and prostate cancer risk was "limited-no conclusion": the summary relative risk was 1.00 (95% CI: 0.99, 1.01) per 100 IU/day for supplemental vitamin E based on seven cohort studies. Three clinical trials tested the efficacy of vitamin E in the prevention of prostate cancer. One trial reported that γ-tocopherol supplements protected against prostate cancer among male heavy smokers in Finland [228]. However, protection against prostate cancer was not a prespecified hypothesis in the trial. A later intervention trial of vitamins E (400 IU/alternate days) and C (500 mg/day) in 14,641 male physicians conducted in the United States provided no support for either an immediate or a long-term benefit of vitamin E supplements in the prevention of prostate cancer: relative risk was 0.97 (95% CI: 0.85, 1.09) during the intervention period [229] and 0.99 (95% CI: 0.89, 1.10) in the posttrial follow-up [230]. In another intervention trial of vitamin E (400 IU/day) and selenium (200 μg/day) among 35,533 healthy men, there was a statistically significant increase in risk (relative risk = 1.17; 95% CI: 1.004, 1.36) in the vitamin E group, suggesting that vitamin E supplementation actually increases the risk of prostate cancer among healthy men [231]. Thus, the weight of the existing epidemiological evidence on vitamin E intake, either dietary or through supplementation, does not support a protective effect against prostate cancer, though the data showing increased risk associated with low plasma levels of γ-tocopherol are suggestive. It is notable, however, that one intervention trial suggested that vitamin E supplementation may actually be harmful [231], especially among men with low selenium status [232].

Vitamin E inhibits prostate carcinogenesis in rats and mice [233,234] and the growth of human prostate cancer cells in nude mice [235–237]. Possible cancer prevention mechanisms include antioxidative and antiinflammatory activities, modulation of nuclear receptors, inhibition of cell growth, and induction of apoptosis [238].

c Carotenoids (β-Carotene and Lycopene)

The epidemiologic evidence related to β-carotene and prostate cancer is substantial and consistently null. Thus, the CUP report reached a firm conclusion that a substantial effect of β-carotene, either through food or supplements, on the risk of prostate cancer is unlikely. None of the 11 cohort studies identified in the SLR reported a statistically significant association when comparing the highest versus the lowest categories of dietary β-carotene. In the dose–response meta-analyses in the SLR, the summary risk estimate was 1.00 (95% CI: 0.99, 1.00) per 700 μg of dietary β-carotene per day based on 10 cohort studies. In addition, the SLR included three intervention trials of β-carotene supplements, none of which reported a significant effect on prostate cancer risk. The meta-analysis in the SLR also showed no significant association of β-carotene in prediagnostic blood with prostate cancer (summary risk estimate = 0.99; 95% CI: 0.95, 1.04) per 10 μg/100 mL based on nine prospective studies. Since that SLR, a Japanese cohort study found no association of β-carotene intake with prostate cancer [174].

A carotenoid of particular interest with regard to prostate cancer is lycopene, found primarily in tomatoes and tomato products (other food sources include watermelon, grapefruit, and guava). The CUP report placed lycopene

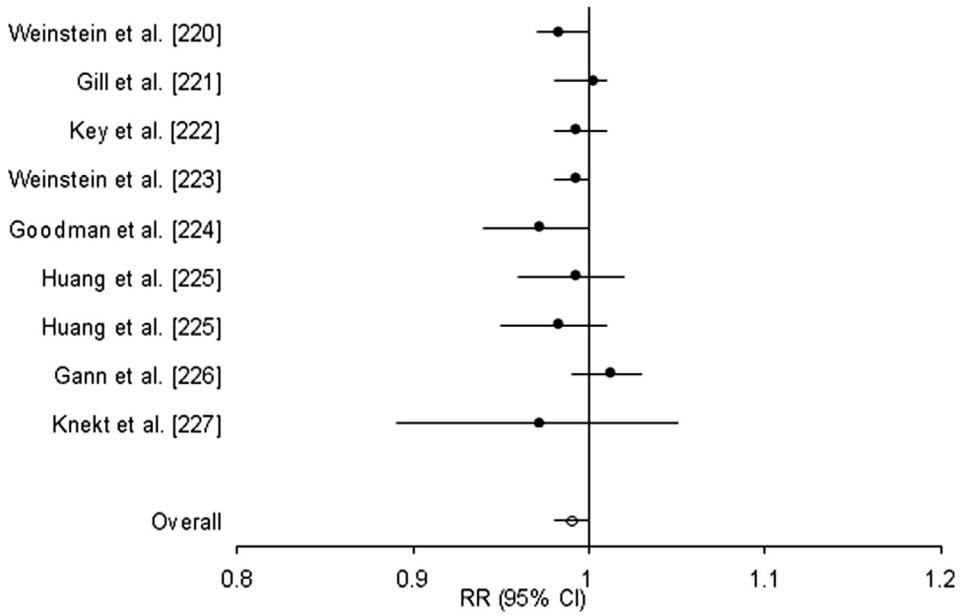

FIGURE 35.3 Circulating α-tocopherol and prostate cancer in prospective studies. *RR*, relative risk, per 10 μg/L; error bars indicate 95% CIs. *Adapted, with permission of the World Cancer Research Fund International, from World Cancer Research Fund International, Systematic literature review. The associations between food, nutrition and physical activity and the risk of prostate cancer. Available from: <http://wcrf.org/int/research-we-fund/continuous-update-project-findings-reports/prostate-cancer>, 2014.*

in the category "limited-no conclusion" due to limited evidence. The meta-analysis in the SLR yielded a nonstatistically significant summary relative risk estimate of 0.98 (95% CI: 0.93, 1.02) for dietary lycopene based on seven cohort studies, and 0.99 (95% CI: 0.79, 1.09) per 1 serving/day for tomatoes based on seven cohort studies [52]. In addition, a summary risk estimate based on 10 cohort studies that measured lycopene in blood was 0.99 (95% CI: 0.96, 1.01).

Since that SLR, one cohort study reported no association with dietary lycopene [95], while another cohort study reported that increasing tomato sauce intake was associated with a decreased risk of prostate cancer [239].

β-Carotene, lycopene, and other carotenoids are widely distributed in human tissues, including the prostate [240,241], where, as potent antioxidants, they help protect cell membranes, DNA, and other macromolecules from damage by reactive oxygen species. Other biological activities of carotenoids, such as the upregulation of gap-junctional communication [242], may also contribute to their anticarcinogenic effects. In three human prostate cancer cell lines (PC-3, DU 145, and LNCaP), β-carotene and lycopene significantly inhibited in vitro growth rates [243,244].

d Selenium

In the CUP, evidence for selenium supplements was too limited to draw any conclusions. In an early randomized intervention trial of a daily selenium supplement (200 μg) for men with a history of skin cancers, an incidental finding was a lower incidence of prostate cancer in men who received the intervention [245]. However, a large randomized intervention trial using supplemental selenium (200 μg/day) and vitamin E (400 IU/day) provided no evidence in support of a beneficial effect of selenium on prostate cancer risk [246]. A recent updated analysis from this trial reported that selenium supplementation increased the risk of high-grade prostate cancer among men with high selenium status at baseline [232]. Another randomized intervention trial of supplemental selenium (200 μg/day) in men at high risk of prostate cancer failed to support the hypothesis that selenium protects against prostate cancer [247]. Thus, there is no basis for recommending selenium supplementation to reduce prostate cancer risk.

On the other hand, the CUP report concluded that the evidence for an association of low plasma selenium concentrations with an increased risk of prostate cancer is limited, but suggestive. The SLR identified 17 relevant studies, and 7 reported an inverse association when comparing the highest versus the lowest categories. In the dose–response meta-analyses in the SLR, summary risk estimates for selenium in blood were 0.95 (95% CI: 0.91, 1.00) for total prostate cancer based on nine cohort studies (Fig. 35.4) and 0.95 (95% CI: 0.89, 1.00) for advanced cancer based on five cohort studies.

Since that SLR, a Danish cohort reported that plasma selenium was not associated with total or advanced prostate cancer risk, but higher selenium levels were associated with a lower risk of high-grade disease [256]. A recent meta-analysis of five studies showed a decreased risk of prostate cancer associated with higher toenail selenium concentrations (pooled odds ratio = 0.97, 95% CI: 0.95, 0.99 per 0.1 μg/g) [257].

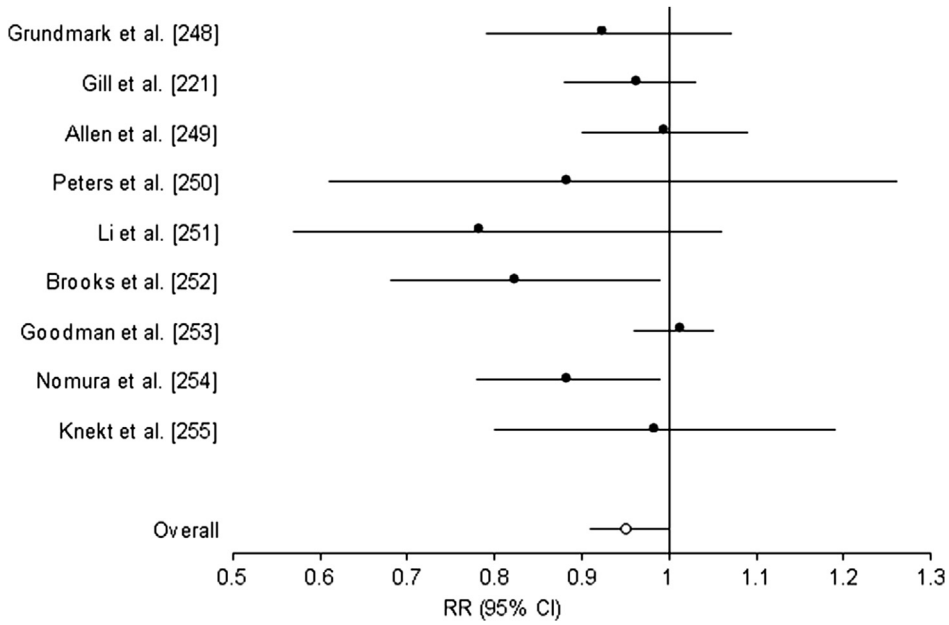

FIGURE 35.4 Circulating selenium and prostate cancer in prospective studies. *RR*, relative risk, per 10 μg/L; error bars indicate 95% CIs. *Adapted, with permission of the World Cancer Research Fund International, from World Cancer Research Fund International, Systematic literature review. The associations between food, nutrition and physical activity and the risk of prostate cancer. Available from: <http://wcrf.org/int/research-we-fund/continuous-update-project-findings-reports/prostate-cancer>, 2014.*

Selenium is a component of glutathione peroxidase, an important enzyme in certain antioxidative pathways. In in vitro experiments, selenium was shown to inhibit the growth of human prostate carcinoma cells [258,259]. Selenium may exert its anticancer effects through any of several proposed mechanisms, such as antioxidation, enhanced immune function, inhibition of cell proliferation, and induction of apoptosis [259].

e Isoflavonoids

The potential role of isoflavonoids in prostate carcinogenesis was discussed in the section on legumes.

8 Diet-Associated Protective Factors

a Physical Activity

The role of physical activity in human prostate carcinogenesis is unclear. Due to limited evidence, no conclusion on physical activity was reached in the CUP report. In the SLR, a summary relative risk estimate for the highest versus lowest level of physical activity was 0.97 (95% CI: 0.90, 1.04) for total physical activity based on 10 cohort studies, 0.87 (95% CI: 0.80, 0.95) for occupational physical activity based on 13 cohort studies, and 0.97 (95% CI: 0.90, 1.04) for recreational physical activity based on 21 cohort studies. Since that SLR, two cohort studies found no significant association for total [95,157], occupational, or recreational physical activity [157].

Because exercise influences androgen levels in the body, an effect of physical activity on prostate cancer risk is biologically plausible. Exercise lowers testosterone in the blood, and it also raises the level of sex hormone-binding globulin, which reduces the circulating free testosterone levels; both effects would be expected to lower prostate cancer risk [260–262].

IV GENETICS AND GENE–ENVIRONMENT INTERACTIONS

As noted previously, prostate cancer has a tendency to aggregate in families, and men whose fathers or brothers have had prostate cancer are at a two- or threefold increased risk of getting the disease compared to men without such a family history. The mapping by linkage studies of rare highly penetrant genes that could explain at least part of this familial aggregation identified a number of regions, including several loci on chromosomes 1 and X, but the results were not definitive [263,264].

Because diet (and other behaviors, such as physical activity [265]) can influence androgen, vitamin D, and IGF-I levels in the body, interactions may occur between dietary exposures and inherited susceptibilities in determining the actual risk for prostate cancer. Using linkage- and association-based strategies, many studies of single nucleotide polymorphisms in candidate genes have been reported, with largely disappointing results with regard to both the main effects of the genes and interactions between the genes and other exposures ("gene–environment interactions"). During the last decade, the research focus has been on genome-wide association studies (GWAS), which take an agnostic approach and require large consortial efforts to obtain sufficient numbers of cases. To date, the latter studies have identified approximately 100 distinct prostate cancer susceptibility loci associated with small differences in the risk of this disease [266,267]. One particular region on chromosome 8 (8q24) has been the source of considerable current research activity [268]. Unfortunately, overall, the GWAS-identified loci explain only a small portion of the variability in prostate cancer susceptibility [269].

V CONCLUSIONS AND IMPLICATIONS FOR PREVENTION AND TREATMENT

Considering the combined evidence from descriptive epidemiologic studies (especially the remarkable changes in migrant populations), analytic epidemiologic studies in widely varying populations, experimental studies in animals, and in vitro studies, the likelihood that certain dietary components or general patterns of eating influence the risk of prostate cancer remains high. However, no specific relationships have been established conclusively. Research on this topic should be continued because diet is a modifiable risk factor and because prostate cancer incidence is extremely high in many populations. In addition, further research on genetic polymorphisms that affect susceptibility to prostate cancer, and their possible interactions with dietary risk factors, may help identify high-risk subgroups of men who can be targeted for future preventive programs.

Currently, the primary treatment modalities for prostate cancer consist of surgery, radiation, and hormonal therapy. The fact that the findings for some dietary factors (e.g., saturated fat, well-done meat, calcium) were stronger in advanced or metastatic cases of prostate cancer implies that dietary effects can occur very late in the disease process [270]. This suggests that dietary interventions have the potential not only to reduce the incidence but also to improve the survival rates of the disease, providing another possible treatment modality. Indeed, one study of prostate cancer patients showed significantly worse survival for men in the upper tertile of saturated fat intake (>13.2% of calories) compared with men in the lowest tertile (<10.8% of calories) [271], whereas another study found that survival was improved in patients who consumed higher amounts of tomato products [272]. Explanations for such observations might be a beneficial effect of lower fat intake on circulating

androgen levels [273] or an inhibitory effect of lycopene on tumor growth rates [244].

Based on current knowledge, it is not prudent to make very specific dietary recommendations to prevent or treat prostate cancer. However, taken as a whole, the evidence is consistent with a diet that emphasizes vegetables, and is moderate or low in the consumption of processed meat and dairy products.

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Chapter 36

Nutrition and Colon Cancer

Daniel D. Gallaher[1] and Sabrina P. Trudo[2]
[1]University of Minnesota, St. Paul, MN, United States, [2]University of Arkansas, Fayetteville, AR, United States

I INTRODUCTION

Colorectal cancer is the third most common cancer and third leading cause of cancer death in both men and women in the United States [1]. Approximately 71% of new cases arise in the colon and 29% in the rectum [1]. Epidemiologic evidence from migrant populations suggests there are some modifiable environmental risk factors, such as diet, in the etiology of colorectal cancer [2]. Hence, extensive research has probed the relationship between dietary components and altered colorectal cancer risk. Given that the majority of cases arise in the colon and evidence suggests some differences in etiology between colon and rectal cancers [3–5], this chapter will focus on the emerging evidence of dietary impacts on the risk of colon cancer.

Many approaches have been developed to examine how diet influences risk of colon cancer. Broadly, these include case–control studies, prospective cohort studies, intervention trials using putative intermediate markers of colon cancer risk, animal studies, and cell culture studies. Each approach has advantages and limitations. Epidemiologic studies, such as case–control and cohort studies, are observational studies that primarily demonstrate associations between two variables (e.g., a particular dietary component or dietary pattern and colon cancer risk). Observational studies are thus most useful for generating hypotheses or providing support to findings from intervention or animal studies. However, since they directly examine humans consuming their normal diets, epidemiologic studies are invaluable in identifying potentially beneficial or harmful foods or dietary patterns. Prospective cohort studies are typically considered stronger than case–control studies due to less susceptibility to recall and selection bias. In addition, data from multiple cohort and case–control studies are often combined to yield statistically stronger conclusions due to the combined larger number of study subjects, among other strengths of this approach; however, a primary drawback is that not all published cohort and case–control studies on a given topic provide sufficient data to be included in the meta-analysis.

Another approach for examining the role of nutrition in the etiology of colon cancer is the intervention trial. The advantages of these studies are that foods or dietary patterns being studied are well controlled and there is no question about applicability to humans. However, this approach requires an outcome measure other than the development of cancer, since trials are necessarily of shorter time than the induction period for colon cancer. Currently, there is no unequivocally validated intermediate marker for colon cancer. Recurrence of colon adenomas (polyps) after their removal has been used, but studies of polyp recurrence are long and expensive. Further, even polyps are not completely validated as a marker of colon cancer risk. However, there is reason for optimism, as some studies suggest the possibility that molecular markers collected from either rectal swabs [6] or fecal samples [7] may provide the long sought validated marker of elevated risk of colon cancer.

Animal studies represent a complementary approach that allows a more mechanistic examination to the study of diet and colon cancer. Although questions about the applicability of findings from animals to the human situation must continually be considered, there is no doubt that findings from animal studies provide insight into colon cancer in humans. Animal studies are often the best approach to examine how consuming different dietary components influence initiation events such as biotransformation of carcinogens, DNA adduct formation, DNA repair, and apoptosis, as well as postinitiation events such as changes in signaling pathways and eventual tumor formation.

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Cell culture studies are frequently employed for the study of how isolated compounds influence cancer cell growth and signaling pathways, among other aspects of carcinogenesis. Nonetheless, they are severely limited in that whole foods requiring digestion cannot be examined nor can food components that are normally metabolized after consumption. Consequently, for the purposes of this chapter, we shall focus on only animal, case–control, prospective cohort, and intervention studies related to nutrition and cancer. In discussion of the animal studies, we shall focus on those studies using whole foods and their effect on morphological endpoints of colon cancer, either adenomas (benign tumors that may progress to cancer), adenocarcinomas (cancerous tumors), aberrant crypt foci (ACF, believed to be precancerous lesions), or mucin-depleted foci (MDF, a subpopulation of ACF which are suggested to be highly dysplastic and are more immediate precursors to tumors than ACF) [8]. A representative image of ACF and MDF is shown in Fig. 36.1. For discussion of human studies, we will focus similarly on studies using colon cancer as the endpoint, with the exception of intervention trials. Emphasis will likewise be on studies of whole foods with a few exceptions.

The majority of these studies have investigated diet components that can be grouped into five categories: Fruits, Vegetables, and Legumes; Meats; Milk and Dairy Foods; Whole Grains; and Beverages. For each category we will summarize the proposed impact on colon cancer risk, including putative biological mechanisms for influencing cancer risk, and review the relevant animal and human data. Fig. 36.2 provides a representation of the process of carcinogenesis, and will provide a basis for understanding at what point(s) different diet components can influence this process.

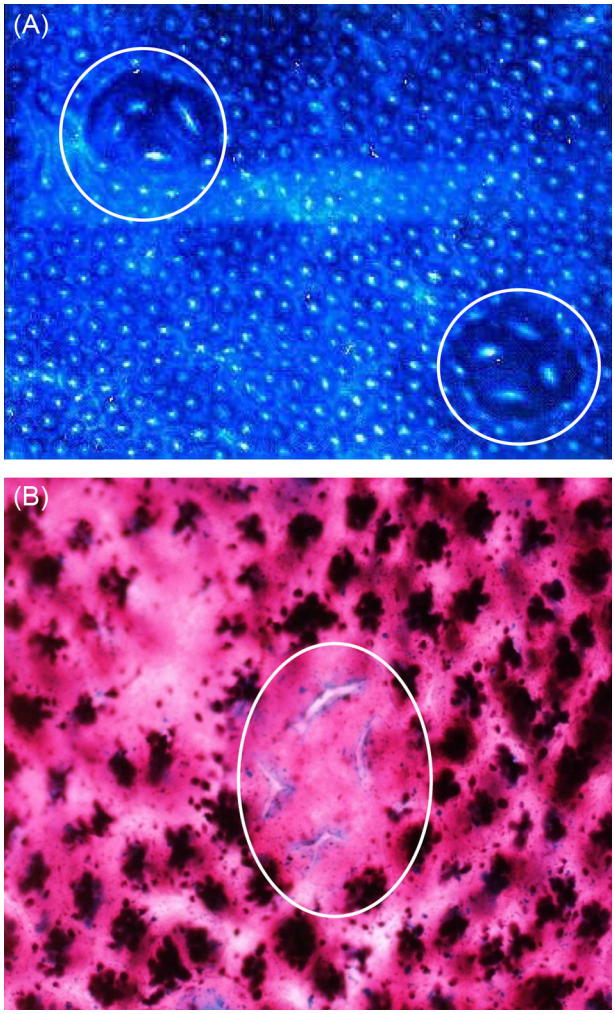

FIGURE 36.1 (A) Aberrant crypt foci (ACF) and (B) mucin-depleted foci (MDF) in rat colon. ACF are visualized by staining whole mounts of colon with methylene blue. MDF are ACF that show no mucin staining using high-iron diamine alcian blue. Normal crypts stain black.

II FRUITS, VEGETABLES, AND LEGUMES

Proposed mechanisms for influencing cancer risk. It has been hypothesized that plant foods protect against cancers such as colon cancer [9]. Thousands of phytochemicals have been identified in fruits, vegetables, and legumes, many of which are capable of modulating various processes related to colon cancer development. For example, apoptosis (or programmed cell death) is a means by which cells with DNA damage can be safely eliminated instead of becoming cancerous. Flavonoids that are found in many fruits and vegetables induce apoptosis in a variety of models [10,11]. Other phytochemicals that induce apoptosis include proanthocyanidins (apples, chocolate, grapes, berries, other fruits), resveratrol (grape skins, peanuts), isothiocyanates (derived from cruciferous vegetables like broccoli and cabbage), and limonene (citrus fruits, cherries) (reviewed in Refs. [10,12]).

Second, many of the naturally occurring compounds in plant foods also interfere with oxidative processes by acting as antioxidants or increasing antioxidant activity. Antioxidants inhibit or mitigate the damage to cells from reactive oxygen species (ROS) that can lead to carcinogenesis. Compounds shown to be antioxidants or to increase antioxidative activities include vitamin C, vitamin E, provitamin A and other carotenoids, flavonoids, proanthocyanidins, isoflavonoids (soy), isothiocyanates and indoles (cruciferous vegetables), and resveratrol (see reviews [10,12,13]).

A third major process modulated by phytochemicals is the metabolism of carcinogens. Several groups of biotransformation enzymes metabolize carcinogens by typically first exposing functional groups on the parent compound, referred to as phase I metabolism and, secondly, conjugating the metabolite with another molecule, called phase II metabolism. The net effect is usually a safer

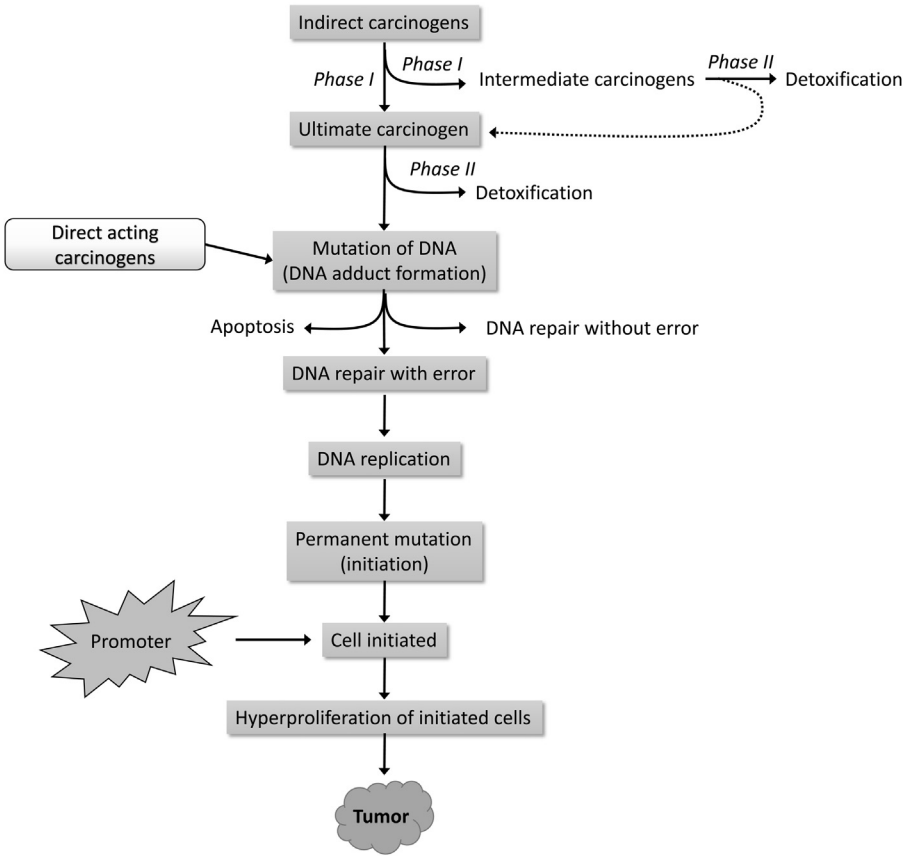

FIGURE 36.2 An overview of the pathway of chemical carcinogenesis. Indirect carcinogens can be detoxified through phase I and phase II metabolism, although for a few compounds, phase II metabolism can lead to carcinogen activation (dotted line). However, some indirect carcinogens are activated by phase I metabolism, leading to mutations in DNA through formation of DNA adducts. DNA repair mechanisms can remove the adducts. If mutation is extensive, this can result in cell death through apoptosis. However, if the DNA is not repaired correctly, and DNA replication occurs, this can lead to a permanent mutation, forming an initiated cell. Additional mutations or promoters of cell proliferation can lead to hyperproliferation and potentially a tumor. *Adapted from G.A. Belitsky, M.G. Yakubovskaya, Genetic polymorphism and variability of chemical carcinogenesis, Biokhimiya 73 (2008) 675–689, http://www.protein.bio.msu.ru/biokhimiya/contents/v73/full/73050675.html.*

and water soluble product that can be excreted. The cytochrome P450s (CYPs) are generally involved in the first step and the second step is mediated by conjugating enzymes such as glutathione *S*-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), *N*-acetyltransferases, and sulfotransferases (SULTs). However, carcinogen metabolism by these collective enzymes is complex, in that the enzymes have broad substrate specificities and in some instances actually toxify the substrate instead of detoxifying it by the chemistry they mediate (i.e., activate procarcinogens). Given the number of phytochemicals that modulate biotransformation enzyme expression and activity, a widely investigated hypothesis is that diet could optimize biotransformation activity toward net detoxification of carcinogens. For example, CYP activity is influenced by isothiocyanates, furanocoumarins, and phenolic compounds [14,15]; GST activity is modulated by isothiocyanates and cruciferous vegetables [15,16]; many flavonoids, isoflavonoids, polyphenols, and some carotenes modulate UGT activity [17]; and evidence indicates that flavonoids and isoflavonoids inhibit several SULTs [18].

A fourth cancer-related process that is modulated by phytochemicals is inflammation. During promotion, cytokines and chemokines can serve as tumor growth factors and tumor survivor factors; proinflammatory cytokines can also regulate epithelial-mesenchymal transition and thus influence invasion and metastasis [19]. Flavonoids, proanthocyanidins, isothiocyanates, and resveratrol have demonstrated antiinflammatory activity [12,19].

Additionally, many plant foods are rich in folate, a water soluble B-vitamin. Folate aids in methylation of DNA, and methylation patterns are key in epigenetic regulation of gene expression. Finally, fruits, vegetables, and legumes provide fiber which may prevent colon cancer by increasing bulk of stool, decreasing transit time through the gut, and diluting carcinogens [20].

Animal studies. Few studies have examined the effect of whole fruits on colon carcinogenesis. Carcinogen-treated rats fed freeze-dried blueberries, blackberries, plums, or mangos, at 5% of the diet, had large reductions in ACF compared to the control group [21]. A similar finding was reported with freeze-dried black raspberries [22] and with whole apples [23]. Although feeding dried plum powder, produced by air drying, did not result in a reduction of ACF [24], feeding a puree of dried plums to carcinogen-treated rats did result in a highly significant reduction of ACF [25]. Thus, studies of fruit feeding have been mostly consistent in showing a reduction in colon cancer risk, although the manner of preparation of the fruit may be important.

Of the vegetables, cruciferous (*Brassica*) vegetables, such as cabbage, broccoli, Brussels sprouts, and cauliflower, have received the most attention for their chemopreventive properties. Cruciferous vegetables contain glucosinolates, which are hydrolyzed by the plant enzyme myrosinase after tissue damage, such as by chopping or chewing, to isothiocyanates and indoles, which evidence suggests are the active agents. Cruciferous vegetables fed to carcinogen-treated animals result in significant reductions in ACF [26]. Further, a tendency for reduction in ACF has been found with juices of garden cress [27] and Brussels sprouts [28], but not red cabbage [28]. Finally, carcinogen-treated mice that were fed cabbage had fewer adenomas, a benign tumor that can progress to a cancerous tumor [29]. Allium vegetables, such as garlic and onion, have also been examined. This family of vegetables is notable for high concentrations of organosulfur constituents, such as diallyl disulfide and *S*-allylcysteine. Studies of carcinogen-treated rats fed garlic have shown a reduction in either ACF [30] or MDF [31], as well as a reduction in tumor incidence [32]. Aged garlic extract reduced large ACF (>4 aberrant crypts/ACF) [33]. Aged garlic extract is prepared by prolonged extraction of fresh garlic, and is less irritating than fresh garlic. Dried onions also reduced ACF in carcinogen-treated rats [34]. Thus, vegetables from different botanical families, containing very different profiles of phytochemicals, appear to be chemopreventive in animal models.

Legumes commonly consumed by humans include soy, beans, peas, lentils, and peanuts. Of these, soy has received the most attention for colon cancer prevention due to evidence that isoflavones present in soy, which have phytoestrogenic activity, may be chemopreventive. Soy, however, is essentially never consumed as the whole bean. It is consumed as a myriad of processed products, including soy flour or protein isolates, tofu, and fermented forms such as tempeh and miso. Soy protein isolate fed to carcinogen-treated rats has reduced tumor incidence [35] and ACF number [36,37]. Soy flour has also reduced ACF number [38]. Interestingly, miso had no effect on ACF number or tumor incidence [39]. However, in contrast, a fermentation product of soybean, black bean, and green bean was found to reduce the growth of tumors resulting from injection of CT-26 colon cancer cells [40]. Few other legumes have been examined. In carcinogen-treated rats, garbanzo bean flour [38] and lentils [37] reduced ACF number whereas cooked navy beans had no effect on tumor incidence [41]. Thus, the evidence suggests that soy, as either a protein isolate or whole flour, is chemopreventive in animal models; too few studies have been reported to be confident about the effect of other legumes.

Human studies. While in vitro studies, animal studies, and many human intervention trials suggest mechanisms that are biologically plausible, cohort and case–control studies have been inconsistent regarding protection against colon cancer by plant foods. Most studies through the 1990s reported 30–40% reduction in risk in those with the highest vegetable intake relative to those with the lowest intake [9,42–46]. Accordingly, the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) reported in 1997 that, after critical review of the research literature by experts, the evidence for protection against colon cancer by diets rich in vegetables was "convincing" [47]. Subsequent studies, however, were less supportive. For example, the European Prospective Investigation into Cancer and Nutrition (EPIC) study observed in 2009 an inverse association for usual combined fruit and vegetable intake and colon cancer, however with adjustment for total fiber the risk estimate for colon cancer lost significance when comparing the highest with the lowest quintile of intake [48]. The reassessment of the evidence by the WCRF/AICR published in the 2011 Continuous Update Project (CUP) report for colorectal cancer indicated limited-suggestive evidence for risk reduction by fruits and nonstarchy vegetables [49]. Nevertheless, the 2011 CUP report does conclude that there is convincing evidence that foods containing dietary fiber do decrease the risk of cancers of the colon and rectum. Studies investigating the independent association of fruit and vegetable intake with colon cancer since the 2011 CUP have continued to report inconsistent results. After additional investigation in 2015 with longer follow-up within EPIC, study investigators reported that although there was suggestion of lower colon cancer risk with increased fruit and vegetable intake, the study did not support a clear inverse association [50]. EPIC was also used to assess the association with colon cancer of plasma concentrations and dietary intakes of carotenoids and vitamins A, C, and E which are generally found in high levels in fruits and vegetables. The authors concluded that although inverse associations with colon cancer were suggested, all of the inverse associations lost statistical significance after adjusting for multiple comparisons except for retinol [51]; the authors further suggested that the possible inverse association between fruit and vegetable intake and colon cancer may be due to compounds other than those investigated in the study. In the Shanghai Men's Health Study, a cohort of over 60,000 men, study investigators reported an inverse association between fruit intake and colon cancer, little evidence for an association between vegetable consumption and colorectal cancer, and an inverse association for legume intake [52]. Similarly, a systematic review and meta-analysis of cohort and case–control studies in the Japanese population found insufficient evidence of an association between vegetable intake and colorectal

cancer risk [53]. Other investigators have pursued more targeted assessment of the intake of specific vegetables. For example, Wu et al. [54] conducted a meta-analysis that included data from 24 case–control and 11 prospective studies to investigate the association of cruciferous vegetable intake and colon cancer risk; statistically significant inverse associations were observed particularly for distal colon cancer in both case–control and prospective studies. Likewise, the meta-analysis by Tse and Eslick [55] that included 33 studies also showed an inverse association between cruciferous vegetable consumption and colon cancer, particularly with broccoli. The 2011 CUP report concluded that garlic probably protects against colorectal cancer [49]; however, no evidence of a protective association was found in a 2014 meta-analysis that included studies specifically investigating allium vegetables (onion, garlic, leeks, etc.) [56].

In sum, the human data on fruits, vegetables, and legumes intake reducing colon cancer risk is inconsistent. The inconsistencies could be related to study design differences in population-based studies such as inconsistent discrimination between effects on proximal versus distal colon, low sample size and case numbers, low or narrow range of intake of plant-based foods in the population studied, types of plant foods consumed in different populations studied, and error in measuring dietary intake. Additionally, a possible explanation for existing discrepancies between animal data and population-based data is that animal studies frequently use purified phytochemicals as the interventions and there may be differences in net effects between phytochemical treatment versus treatment with the intact food source (see review by Liu [57]). Plant foods contain thousands of bioactive constituents that may interact or counteract each other as normally consumed in whole foods and complex diet patterns. Nonetheless, that individual phytochemicals show promise mechanistically in animal and in vitro studies gives impetus for continued work in identifying the role of plant foods in colon cancer prevention (Fig. 36.3).

III MEAT

Proposed mechanisms for influencing cancer risk. There are several components of meat whose consumption can plausibly be linked to enhancing the risk of colon cancer. Cooking meat at high temperature causes formation of heterocyclic aromatic amines (HAAs), which are known carcinogens in rodents [58]. HAA consumption produces chemical modifications of DNA [59], known as DNA adducts. The structures of two HAAs commonly found in foods and their DNA adducts are shown in Fig. 36.4. Formation of DNA adducts is generally considered to be a "necessary but not sufficient" event for tumor formation [60]. Although the failure to detect differences in colon cancer risk between populations consuming well-done meat versus normal meat [61] has caused some to question the role of HAAs in human colon carcinogenesis, a recent study in which dietary HAA intake was estimated for three HAAs found significant positive correlations between intake of several HAAs and colorectal tumors [62]. Thus, whether the HAAs present in grilled meat represents a colon cancer risk in human remains an open question. A second meat component suspected of increasing risk of colon cancer is heme, present in myoglobin, hemoglobin, and various heme proteins, which is suggested to act as a colon cancer promoter [63]. A large cohort study, in which heme iron intake was found to be significantly associated with an increased risk for tumors that carried G>A mutations in the APC gene [64], suggests a mechanism. Since G>A mutations are characteristic of

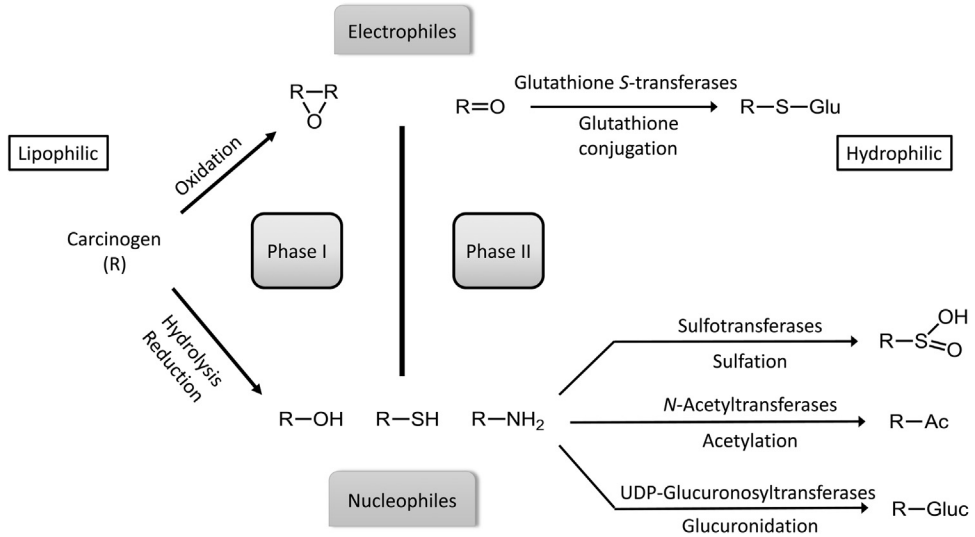

FIGURE 36.3 An overview of phase I and phase II metabolism. Carcinogens can undergo oxidation reactions that allow conjugation with glutathione by GSTs. Alternatively, carcinogens can undergo hydrolysis or reduction reactions, and subsequently be sulfated by SULTs, acetylated by *N*-acetyltransferases, or glucuronidated by UGTs. The result is a more hydrophilic compound that can be excreted in the bile or urine. *Adapted from https://commons.wikimedia.org/wiki/File:Xenobiotic_metabolism.png.*

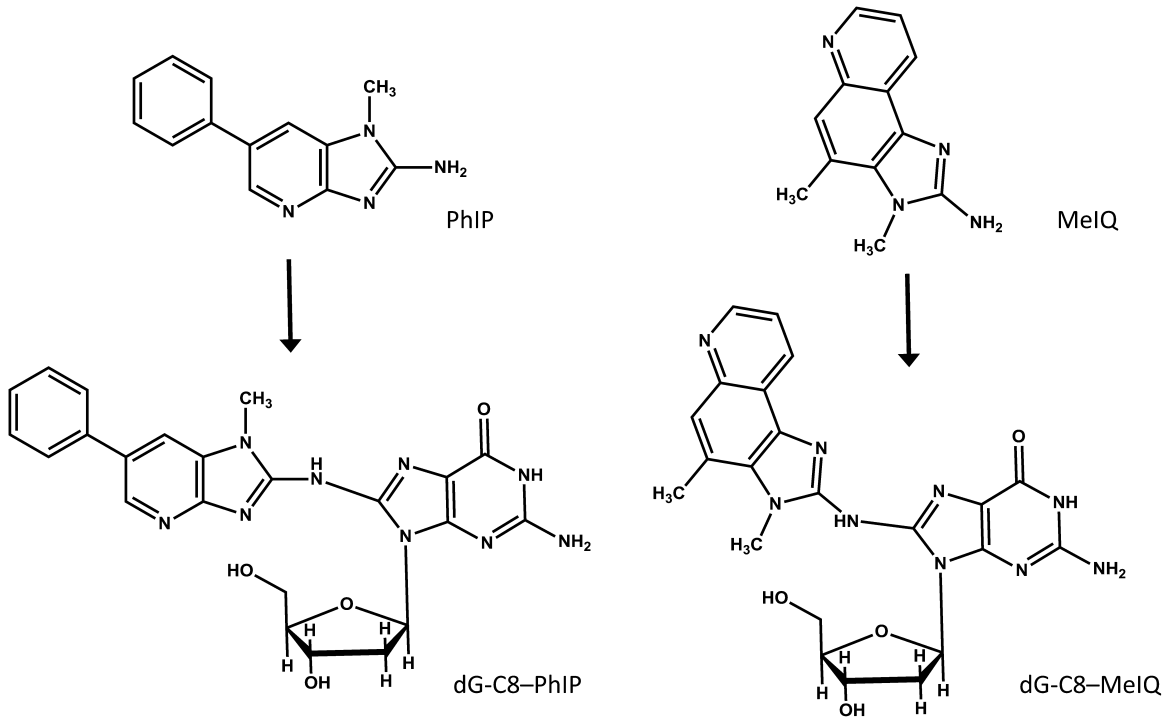

FIGURE 36.4 Structure of two foodborne HAAs, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) and 2-amino-3,4-dimethylimidazo[4,5-f] quinolone (MeIQ) and their DNA adducts *N*-(deoxyguanosin-8-yl)−PhIP (dG-C8−PhIP) and *N*-(deoxyguanosin-8-yl)−MeIQ (dG-C8−MeIQ).

DNA alkylating agents [65], heme may promote mutagenesis. However, in a study in which mice were fed heme-containing or heme-free diets for 18 months, no difference in either DNA alkyl adducts, such as O^6-methyl-2-deoxyguanosine, or tumor number were found between the groups [66]. Thus, a mechanism by which heme may increase colon cancer risk remains uncertain. Nitrite and *N*-nitroso compounds (NOCs) represent yet another class of compounds that are present primarily in processed meats (e.g., grilled bacon) and smoked fish, and have been shown to be carcinogenic in animal studies [67]. Further, nitrates and nitrites, which can give rise to NOCs endogenously, have been classified as probable human carcinogens by the International Agency for Research on Cancer (IARC) [68]. Finally, animal sources of dietary fat, primarily saturated fat, are implicated as a risk factor in epidemiologic studies [69,70]. How saturated fat may promote colon carcinogenesis remains very unclear. However, carcinogen-treated rats fed beef tallow were reported to have greater expression of β-catenin (part of the Wnt signaling pathway) and decreased apoptosis in the colonic mucosa [71], both of which are associated with greater colon cancer risk. Thus, saturated fat may shift intracellular signaling pathways toward a condition of greater cancer risk.

Animal studies. A large number of animal studies of the effect of red meat on colon carcinogenesis have been conducted. These include studies where the endpoints were either colonic tumors [72−74] or ACF [75] and where either carcinogen-treated animals or genetic models [76] of colon carcinogenesis were used. Overall, these studies do not support an effect of red meat on promoting colon carcinogenesis, and in some cases beef was protective [73,74,76]. For example, diets containing either 30% or 60% of freeze-dried beef, chicken, or bacon were fed to carcinogen-treated rats and ACF number determined after 100 days of feeding. These diets were compared to casein-based control diets that used either olive oil or lard as fat sources in order to approximate the fat content of the meat diets. There were no differences in the number of ACF among any of the diets [75]. Pence et al. examined the effect of lean beef versus casein at two levels of dietary fat (5% vs 20%) and two types of dietary fat (corn oil vs tallow) on tumor development in carcinogen-treated rats [74]. After 27 weeks of feeding, total incidence and the number of tumors were lower in the beef-fed rats than the casein-fed rats.

To explain why animal studies do not support a promotional effect of red meat whereas epidemiologic studies largely do (discussed below), Pierre et al. proposed that high calcium diets may protect against the promotional effect of red meat [77]. This was based on the observations that most rodent diets are relatively high in calcium, that heme added to the diets of rats promoted colonic epithelial proliferation [78], and that this heme-induced

proliferation was inhibited by high calcium [79]. This hypothesis was examined in carcinogen-treated rats fed diets containing 60% beef in the context of either a low or high calcium diet. A casein-based diet served as the control. Both ACF and MDF were increased in the low-calcium beef diet compared to the low-calcium casein diet. However, the high-calcium beef diet did not differ in ACF or MDF from the low-calcium casein group, supporting the hypothesis that calcium suppresses the promotional effect of red meat. Unfortunately, complicating the interpretation of these results was the finding that the high-calcium casein diet had ACF and MDF numbers equivalent to the low-calcium beef diet. The authors suggested that this unexpected finding may be due to the phosphate component of the calcium phosphate used as the calcium source in the diet. Further, a diet of 60% beef represents a concentration of beef in the diet well beyond what would be consumed by humans. In a subsequent study of the interaction of processed meat and calcium on colon cancer risk, carcinogen-treated rats were fed an air-exposed picnic ham containing nitrite (47% of the diet), in the context of a very low or high calcium diet [80]. Rats fed the high calcium diet had significantly fewer colonic MDF than those fed the low calcium diet, although the number of ACF did not vary between the two diets. These two studies suggest an important interaction between dietary red meat and calcium in terms of colon cancer risk that warrants further study.

Human studies. In the 2011 CUP by WCRF/AICR, the evidence was assessed as "convincing" for intake of red meat and processed meat (smoked, cured, salted, etc.) increasing the risk of colon and rectal cancer [49]. Moreover, on behalf of IARC, a working group of 22 scientists from 10 countries assessed over 800 epidemiological studies of the association between colorectal cancer and red meat and processed meat. They concluded in 2015 that consumption of processed meat is "carcinogenic to humans" with the largest body of evidence concerning colorectal cancer; they additionally concluded that red meat intake is "probably carcinogenic to humans" based on limited evidence in humans [81]. They define processed meat as meat that has been transformed (salting, curing, smoking, etc.) for enhanced flavor or improved preservation, and red meat as unprocessed mammalian muscle meat such as beef, pork, lamb, etc. However, other investigators critically reviewing the epidemiologic data on unprocessed red meat and colorectal and colon cancer contradictorily conclude that the data show weak associations, lack a clear dose−response trend, vary by gender, and are susceptible to the collinearity of meat intake with other dietary and behavioral factors which limits isolation of the independent effects of meat [82−84]; they also suggest that data from epidemiologic studies do not support that there is a clear underlying biological mechanism [84]. Interestingly, in a recent analysis of data from two large prospective cohorts (Nurses' Health Study, $n = 87{,}108$ women; Health Professionals Follow-up Study, $n = 47{,}389$ men) processed red meat was associated with higher risk of distal colon cancer while unprocessed red meat was associated with lower risk after adjusting for calcium, folate, and fiber intake [85]; the authors thus concluded that there was little evidence that unprocessed red meat substantially increases risk of colorectal cancer. In numerous prospective cohort and case−control studies of the association between poultry consumption and risk of colon cancer, results quite consistently indicate no association with colorectal or colon cancer risk [86]. This lack of an association has raised suspicion over the role of HAAs as an underlying mechanism of meat and colon cancer risk because, similar to red meat, poultry cooked at high temperatures is also a source of HAA. Potential challenges to finding consistency across human studies of meat and colon cancer may include accuracy in assessing cooking or processing methods of meats and level of doneness of meats (thus HAA exposure). Additionally, genetic polymorphisms, such as in genes involved in metabolism of HAAs or DNA repair, could modify the risk related to a putative mechanism [87−89].

IV MILK AND DAIRY FOODS

Proposed mechanisms for influencing cancer risk. A number of constituents in dairy foods have been investigated for their chemopreventive potential, with calcium and vitamin D having received the most attention. However, lipid components found in dairy fat, such as conjugated linoleic acid (CLA) and sphingolipids, as well as dairy proteins, particularly the whey proteins, have also been studied.

Perhaps the earliest suggestion for the chemopreventive action of dietary calcium was put forth by Newmark et al. [90], who suggested that calcium would precipitate fatty acids and bile acids within the colonic lumen, thereby reducing their ability to irritate the colonic epithelium. This irritation was suggested to be the manner in which they act as cancer promoters. This hypothesis received experimental support from studies showing that dietary calcium decreased the solubility of fatty acids and bile acids in the large intestine [91] and thereby reduced the cytotoxicity of the fecal water [92]. The calcium sensing receptor, which is involved in controlling differentiation of colonic epithelial cells, may also play a role. In cell culture studies, calcium increased transcriptional activity of the calcium sensing receptor and induced a less malignant phenotype in colon cancer cells, an effect also noted with $1,25(OH)_2D_3$, the active form of vitamin D [93].

Since the active form of vitamin D functions as a steroid hormone, functioning as a transcription factor bound

to the vitamin D receptor (VDR), it is understandable that the proposed mechanisms of action of vitamin D involve effects on gene expression. Since many sporadic colon cancers show mutations in the adenomatous polyposis coli gene (*Apc*) [94], several studies have examined the role of the active form of vitamin D on pathways related to *Apc*. Inactivation of *Apc* results in activation of the WNT pathway and accumulation of β-catenin in the nucleus which, through a complex series of events [95], leads to constitutive activation of target genes promoting proliferation of colonic epithelial cells. Subsequent mutations are thought to lead to tumor development. In mice with mutations in *Apc*, those also carrying a mutation in *VDR* accumulated more nuclear β-catenin [96], suggesting that 1,25-$(OH)_2D_3$ acts to modulate the WNT pathway. Severe deficiency of 1,25-$(OH)_2D_3$, created by knocking out 25-hydroxyvitamin D 1α-hydroxylase, the enzyme responsible for its formation, has been shown to induce significant colonic inflammation [97]. Chronic inflammation in the colon has long been known to increase colon cancer risk, as illustrated by the increased risk of colon cancer in patients with ulcerative colitis [98]. Whether the mild to moderate degrees of vitamin D deficiency encountered in human populations also result in colonic inflammation remains to be investigated.

CLA is a term for a group of isomers of linoleic acid that contains a conjugated double bond system. Dairy products represent a major dietary source of CLA, with the two major forms being *cis*9, *trans*11-CLA and *trans*10, *cis*12-CLA. Almost all studies examining the chemopreventive mechanisms of CLA have used purified CLA in cell culture studies. No clear mechanism has emerged from these studies. There is some evidence that *cis*9, *trans*11-CLA decreases cyclooxygenase-2 (COX-2) expression in breast cancer cell lines [99], a change associated with reduced cancer risk in the colon. Increased rates of apoptosis are associated with decreased colon cancer risk, and several studies showed induction of apoptosis with CLA [100,101]. Supporting a chemopreventive effect of CLA, a study in carcinogen-treated rats fed bitter gourd seed oil, which contains >50% CLA in the forms of α- and β-eleostearic acids, found statistically significant reductions in ACF and adenomas after 32 weeks of feeding. However, additional studies are necessary to establish the chemopreventive mechanism of CLA.

Sphingolipids are a category of structurally diverse lipids having a sphingoid base with long-chain fatty acids attached in an amide linkage and containing polar head groups. They are present in small amounts in most foods but are abundant in dairy products, particularly cream and cheese [102]. Sphingolipids, along with their digestion products (ceramides and sphingosines), are highly bioactive. A mechanism of chemoprevention by sphingolipids has not been conclusively identified, but ceramides are involved in cancer cell growth, differentiation, and apoptosis [103]; supplementing the diet with sphingolipid increases apoptosis in the colonic epithelium in carcinogen-treated mice [104]. There is also evidence that sphingolipids normalize aberrant Wnt/β-catenin signaling [105], a pathway that is frequently dysregulated in colon cancer. Thus, sphingolipid-induced changes in differentiation and apoptosis, as well as Wnt signaling, are likely to be involved in the chemopreventive action of sphingolipids.

Dairy proteins have several distinct properties that make them plausible dietary chemopreventive agents. Caseins, the most abundant group of dairy proteins at 80% of the total, have been shown to bind HAAs [106], which are known carcinogens. A casein hydrolysate was shown to inhibit β-glucuronidase activity [107]. Decreased β-glucuronidase activity has the potential to reduce colon cancer risk, as carcinogens can be inactivated by glucuronidation in the liver and excreted in the bile. However, colonic bacteria express β-glucuronidase activity, which hydrolyzes the glucuronide, releasing the active carcinogen. Inhibiting this bacterial β-glucuronidase activity could thus reduce carcinogen release in the colon. Whey proteins, the second most abundant group of dairy proteins, at 20% of the total, are notable as a rich source of the sulfur amino acid cystine. Feeding whey proteins to rats increases tissue levels of the cysteine-containing tripeptide glutathione [108]. This is significant for two reasons. First, glutathione is a potent intracellular antioxidant and can participate in the elimination of ROS, either directly or as a cosubstrate for glutathione peroxidase, which reduces lipid peroxides. ROS can damage cellular macromolecules including DNA, and high levels of ROS are believed to promote cancer. Second, glutathione is a cosubstrate for GST, an enzyme involved in detoxification of xenobiotics, including carcinogens. Animal studies show an inverse relationship between liver glutathione concentration and colon tumor incidence [72], suggesting that increasing tissue glutathione may be chemopreventive.

Animal studies. Tavan et al. [109] reported that carcinogen-treated rats given a diet containing 30% skim milk had a significant reduction in ACF relative to the control group. However, almost no additional studies have been conducted on milk and colon cancer risk in animal models. The focus has been almost exclusively on milk components, both major and minor. Whey proteins, which constitute approximately 20% of milk proteins, reduce tumor formation in carcinogen-treated rats [110] and partially hydrolyzed whey proteins reduce ACF number compared to casein-fed animals [111].

Another major milk component, milk fat, has been examined as two different fractions—the anhydrous milk fat and the milk fat globule membrane. This latter fraction is a protein−lipid complex, rich in sphingolipids, that surrounds the milk fat globules. In carcinogen-treated rats

the milk fat globule membrane, but not the anhydrous milk fat, reduced ACF number [112], pointing to the potential of sphingolipids as an important chemopreventive compound in milk. This is plausible, as a number of animal studies have demonstrated that sphingolipids show chemopreventive effects [10].

A large number of animal studies have examined the potential for calcium to reduce tumorigenesis. A meta-analysis of the studies through 2005 concluded that high calcium diets reduced tumor incidence in carcinogen-treated animals (Relative Risk = 0.91, $p = 0.03$) [113]. Animal studies conducted since 2005 continue to support a role for high calcium diets reducing carcinogenesis. A high calcium diet (5.2 g/kg diet) reduced ACF number in both mice and rats compared to a low calcium diet (1.4 g/kg diet) [114]. A relatively new animal model of colon carcinogenesis is the so-called new Western-Style diet (NWD), which is low in calcium and vitamin D and high in fat, and also has relatively low levels of folic acid, cysteine, and choline bitartrate. Long-term feeding (e.g., 18 months) of the NWD resulted in intestinal tumor formation, primarily in the large intestine [115]. Using this model, 2 years of feeding the NWD with added calcium and vitamin D resulted in no colon tumors compared to 27% of mice fed the NWD [116]. Since both calcium and vitamin D were added, this study cannot determine if either one alone would have had a comparable effect. Regardless, overall, animal studies strongly support a chemopreventive effect of dietary calcium.

Relatively few animal studies have been reported of the influence of vitamin D, independent of calcium (i.e., when dietary calcium was adequate), on tumorigenesis or precancerous lesion. In rats given the direct acting colon carcinogen *N*-methyl-*N*-nitrosourea and lithocholic acid (which acts as a tumor promoter), there were fewer tumors when 1-(OH)-D_3 was also administered [117]. In the Apc^{min} mouse, a genetic model of colon cancer, intraperitoneal injection of 1,25-$(OH)_2$-D_3, the active form of the vitamin, did not reduce the number of intestinal polyps. However, the total tumor load was significantly reduced compared to the control group that was not administered 1,25-$(OH)_2$-D_3 [118]. In carcinogen-treated rats, administration of 1,25-$(OH)_2$-D_3 prior to administration of the carcinogen reduced tumor formation by 50% [119]. The previously described studies examined supplemental vitamin D. In a study using carcinogen-treated rats, it was found that animals fed a high calcium diet had fewer colonic tumors per rat (tumor multiplicity), compared to a normal calcium diet. However, feeding a high calcium diet that was also vitamin D deficient resulted in the loss of the protective effect of the high calcium diet [120]. These few studies suggest that supplemental vitamin D may reduce colon cancer risk, and that vitamin D is necessary for the chemopreventive effect of high dietary calcium. However, in a recent study using both rats and mice with a defective *Apc* gene (Pirc rat and Apc^{min} mouse), supplemental vitamin D did not alter either tumor number or tumor multiplicity compared to animals given a normal amount of vitamin D in either species [121]. More recently, a similar study was conducted feeding a range of vitamin D concentrations as well as 25$(OH)D_3$ [122]. In addition to enumeration of tumors at the end of the study, the investigators followed tumor development endoscopically. No protection against development of the final number of colonic tumors was found. Thus, the results from animal studies are inconsistent and do not provide strong support for a chemopreventive effect of supplemental vitamin D.

Human studies. Based on evidence primarily from observational studies in Western countries, the WCRF/AICR concluded in the 2011 CUP report that intake of milk and calcium probably decrease the risk of colon cancer [49]; in regards to foods containing vitamin D, their conclusion was that there was "limited-suggestive" evidence for a protective association. In 2012, Aune et al. [123] published the results of their meta-analyses that included data from 19 cohort studies; total dairy intake and milk intake were each inversely associated with colon cancer in both men and women. No associations were observed for cheese or other dairy products. Subsequently, a meta-analysis that included 15 prospective studies reported an inverse association between milk intake and colon cancer risk, but in men only [124]. The authors speculated that observing the association in men only could be related to the higher incidence of colorectal cancer in men compared with women [124]. Consistent with the majority of studies on the association between dietary calcium and colorectal cancer risk, Zhang et al. [125] reported in 2016 that total calcium intake was associated with reduced risk of colon cancer in a large cohort of 88,509 women and 47,740 men. They also observed that results were similar for different sources of calcium (from all foods or dairy products only) and that the inverse association was linear and stronger for distal colon cancer than proximal [125]. Since the publication of the 2011 CUP report, evidence is still limited regarding vitamin D intake and colon cancer risk; a 2015 systematic review and meta-analysis utilizing six studies found inconsistent associations for colorectal cancer and vitamin D supplementation [126].

While not conclusive, the evidence is somewhat consistent for a protective effect from milk and calcium intake. The human data on vitamin D are not consistent and the relationship to colon cancer warrants further investigation.

V WHOLE GRAINS

Proposed mechanisms for influencing cancer risk. Whole grains include a heterogeneous collection of cereals,

including wheat, corn, barley, oats, rye, and rice, as well as less commonly consumed cereals such as sorghum, millet, and triticale. Whole grains represent the intact grain, containing the endosperm, germ, and bran. The endosperm is largely composed of starch with some protein, whereas the bran contains most of the dietary fiber and many compounds thought to be highly bioactive, including phenolic acids, flavonoids, and vitamin E. The germ is rich in vitamins, minerals, and oil, and also contains a variety of antioxidants, including vitamin E.

Unsurprisingly, cereals show great variation in their composition of components thought to have health benefits. For example, oats and barley contain substantial amounts of β-glucans, a viscous and highly fermentable type of dietary fiber, whereas wheat, corn, and rice have little β-glucans. There are similar wide variations in antioxidant capacity among the whole grains. Nevertheless, there are sufficient commonalities among the cereals that it is still useful to consider them as a group in terms of colon cancer prevention.

The dietary fiber from whole grains has long been postulated as providing protection from colon cancer by several different mechanisms. One long-standing hypothesis is that dietary fiber reduces contact of potential carcinogens or procarcinogens with the colon, either by dilution of potential carcinogens or procarcinogens due to fecal bulking or by reducing exposure due to a decreased colonic transit time. Another potential mechanism involves the increased production of short chain fatty acids within the colon due to greater quantities of fermentable substrate for colonic bacteria. Of the short chain fatty acids, butyrate has been of particular interest due to many in vitro studies showing that butyrate inhibits growth of cancer cells, causing normalization of cancer cells, or increases cancer cell elimination by increased apoptosis [127]. Yet another potential dietary fiber mechanism is the promotion of the growth of probiotic bacteria (such as bifidobacteria) by fructans (such as inulin). Although increasing probiotic bacteria in the colon by feeding prebiotics reduces colon cancer risk in animal models [128,129], it appears unlikely that humans consuming a normal cereal-containing diet could consume a sufficient quantity of fructans to significantly increase the colonic bifidobacteria population [130]. Therefore, this particular mechanism of chemoprevention may not be relevant to humans not consuming supplements of fructans.

Another oft-discussed potential mechanism of chemoprevention by whole grains is the delivery of antioxidants from whole grains. There are two issues with regard to this mechanism. First, the ability of antioxidants to reduce colon cancer risk is still in doubt. Trials in humans and animal models of colon cancer do not provide strong support for a reduction in risk by α-tocopherol, the form of vitamin E commonly found in supplements, although a mixture of tocopherols shows some promise [131]. For most other natural compounds present in foods, it is uncertain whether the chemopreventive benefit they provide is due to their antioxidant effect or some other property. The second issue is the often poor bioavailability of compounds in cereals with antioxidant activity. For example, ferulic acid, the major phenolic acid in cereals, displays antioxidant activity in vitro [132], but is almost entirely bound within the cereal matrix [133] and therefore poorly available for absorption. Consistent with this is the finding that in diabetic rats, who exhibit elevated levels of oxidative stress, feeding cereal-based diets had no effect on markers of oxidative stress [134].

A number of other compounds found in cereals have been shown, in purified form, to reduce colon cancer risk. These include phytic acid [135,136], sphingolipids [104], and lignans [137] (compounds with a diphenolic ring structure that have phytoestrogen activity). However, whether these compounds, either alone or in combination, contribute significantly to chemoprevention by cereals is difficult to ascertain, in part due to questions about bioavailability.

Thus, cereals contain a plethora of bioactive compounds that could explain any observed chemopreventive effects. As with other whole foods, determining which compound or combination of compounds is responsible for chemoprevention is a difficult task.

Animal studies. Very few studies have examined the effect of whole versus refined grains on colon cancer risk in animal models. Maziya-Dixon et al. [138] fed red and white flour, in both the whole and refined forms, to mice given a chemical carcinogen. After 40 weeks, mice fed the wheat-containing diets did not differ from the wheat-free control diet tumor incidence. Interestingly, though, mice fed the red wheat diets, regardless of refining state, had significantly lower tumor incidence that mice fed white flour, again regardless of refining state. In other words, it was wheat color, not the state of refinement, which influenced tumor incidence. The importance of wheat color, as opposed to state of refining, was confirmed in a study in carcinogen-treated rats, where feeding red wheat, either whole or refined, reduced colonic ACF relative to white wheat [139]. The importance of wheat color was confirmed and extended in a study where it was shown that carcinogen-treated rats fed refined red wheat had fewer MDF than those fed refined white wheat, when fed only in the late postinitiation stage (when the wheat diets were fed 54 days after carcinogen treatment), as well as less β-catenin, indicating less dysregulation of the Wnt signaling pathway [140]. In another study, whole and refined wheat were fed to rats given HAA as a carcinogen. No difference was found between the groups fed whole and refined wheat in the number of colonic ACF [141], although it should be noted that the number of

ACF per animal was extremely small. Thus, in the few studies investigating wheat, the evidence suggests that it is the color of the wheat, not whether it is whole or refined, that is most important in terms of chemoprevention.

Given the paucity of studies in which whole and refined grains have been directly compared, an alternative is to examine studies in which bran feeding has been investigated. This is an imperfect comparison, as whole grains differ from refined grains by the inclusion of both bran and germ in the whole grain. However, as germ represents only about 2.5% of the whole grain, in the case of wheat, this is likely a useful approach.

The vast majority of studies that used cereal bran examined wheat bran, usually at dietary concentrations of 15–20%. Using carcinogen-treated rats, most have found a reduction in colon tumor incidence [142–150]. A study using Min (multiple intestinal neoplasia) mice, which have a mutated *Apc* gene, similar to the mutation in familial adenomatous polyposis patients, and thus spontaneously develop intestinal tumors, reported fewer tumors after feeding brans of several wheat varieties. The efficacy of tumor number reduction inversely correlated with the orthophenolic content (e.g., ferulic acid) of the wheat from which the bran was derived [151]. In addition, several studies have reported a decrease in ACF in carcinogen-treated rats fed wheat bran, relative to rats fed a fiber-free or low fiber diet [148,152]. A study in carcinogen-treated mice found that a diet of 20% wheat bran reduced adenomas relative to a fiber-free control diet, but had no effect on adenocarcinoma incidence [153]. Several studies have even reported an enhancement in tumor incidence in carcinogen-treated rodents fed wheat bran. Carcinogen-treated mice fed 20% wheat bran, from either soft winter white or hard spring wheat, had a much higher incidence of colon tumors than animals fed a fiber-free diet [154]. Similarly, carcinogen-treated rats fed a 20% wheat bran diet also had a greater number of colonic tumors compared to animals fed fiber-free diet, but this was only observed when the wheat bran was fed during carcinogen administration [155]. Overall, however, studies in carcinogen-treated rodents support a reduction in tumor development with feeding of wheat bran.

Fewer studies have been carried out with brans of cereals other than wheat. Oat bran fed to carcinogen-treated rats resulted in a greater number of colonic tumors in the proximal colon, but not the distal colon, compared to a fiber-free control [156]. In Min mice, however, oat bran feeding had no effect on the development of intestinal tumors [157]. Feeding rye bran to carcinogen-treated rats resulted in fewer colon tumors and fewer ACF, compared to the cellulose-fed control group [158]. However, rye bran fed to Min mice resulted in either no effect [157,159] or an increase in intestinal tumors [160]. Barley bran was shown to reduce tumor incidence in carcinogen-treated rats compared to cellulose-fed control group [143], whereas corn bran increased colon tumor incidence in carcinogen-treated rats [142,161]. Finally, rice bran at 30%, but not 10% of the diet, reduced intestinal tumor number in Min mice compared to a cellulose-fed control group [162], but had no effect in carcinogen-treated rats [142].

Thus, animal studies provide considerable support for protection against colon cancer by wheat, primarily based on studies of wheat bran, although there are indications that the color of the wheat is more important than the state of refinement. For other cereals the studies are highly inconsistent and are too few to ascertain whether they have a chemopreventive effect or may even promote colon cancer.

Human studies. The evidence for an association between cereal grains and colon cancer was reported as limited according to the WCRF/AICR report of 2007 [20]; in the 2011 WCRF/AICR CUP report [49], whole grains are folded into the category of foods containing dietary fiber with the conclusion that there is convincing evidence that foods containing fiber decrease colon cancer risk. Specifically looking at whole grains, CUP meta-analyses showed a 16% decreased risk per three servings per day. Since the 2011 CUP report, Kyro et al. [163] reported that intake of whole-grain products, and specifically whole-grain wheat, was associated with lower incidence of colorectal cancer but not colon cancer in a large Scandinavian prospective cohort. However, in a cohort of Norwegian women whole-grain bread was not associated with colorectal cancer, but there was a weak protective association with proximal colon cancer [164]. Two other studies used plasma alkylresorcinols, a biomarker of whole-grain intake, to investigate the association of whole grains with colon cancer [165,166]. Alkylresorcinols are primarily found in the bran of wheat and rye and their concentrations in plasma have been used as an indicator of short-term and medium-term consumption specifically of whole-grain wheat and rye products. Both studies observed an inverse association between plasma alkylresorcinol concentrations and distal colon cancer incidence [165–167]. In sum, there is evidence that whole-grain wheat and rye may reduce colon cancer risk, that the effect may be specific to distal versus proximal colon cancer, and that use of the alkylresorcinol biomarker may increase precision in estimating risk compared to food frequency questionnaires. Further evidence that grains may influence risk of colon cancer includes studies where an increased risk was observed with intake of refined grains [168–170]. However, it should be noted that whole-grain intake is reported to be associated with various factors that could also be related to decreased risk of colon cancer, such as healthy lifestyle, socioeconomic, and dietary factors [171].

Using putative intermediary biomarkers of colorectal cancer, a few intervention trials have been conducted [172–175].

However, they typically used an isolated grain fraction such as the bran or fiber instead of actual whole-grain foods, or else combined high intake of whole-grain foods with other practices that may have an independent effect (e.g., low fat, high vegetable intakes) and thus make it difficult to assess effects attributable specifically to whole-grain intake.

VI BEVERAGES

Proposed mechanisms for influencing cancer risk. Three types of nonnutritive beverages have been studied extensively for potential protective effects against colon cancer (coffee and tea) and harmful effects (alcoholic beverages). The interest in coffee stems from evidence that coffee components such as dieterpenes (cafestol and kahweol) mitigate the genotoxicity of HAAs [176–178]; increase the activities of enzymes that generally detoxify carcinogens (UGTs and GSTs), decrease the activity of some carcinogen-activating enzymes (*N*-acetyltransferases and SULTs), and decrease HAA-mediated genotoxicity [176–181]. Additionally, cafestol and kahweol have antioxidant properties, and induce γ-glutamylcysteine synthetase (the rate limiting enzyme in glutathione synthesis) [182,183]. Human consumption of Italian-style coffee (or espresso) increases plasma glutathione and unfiltered French press coffee increases glutathione content in colorectal mucosa [184,185]. Moreover, coffee is rich in phenolic acids, flavonoids, and melanoidins, many of which have demonstrated antioxidant properties that can depend on degree of roasting [186–188]. In vitro studies suggest that chlorogenic and caffeic acids found in coffee may decrease cell proliferation, cell invasion, angiogenesis, and metastasis, further supporting the hypothesized chemopreventive potential of coffee [189–193].

Both green tea and black tea have also interested cancer prevention researchers. Theaflavin-2 (black tea polyphenol) exhibits antiinflammatory and proapoptotic activities [194]. Green tea polyphenols inhibit proliferation and invasiveness of colon cancer cells [195], induce apoptosis and demonstrate antioxidant activity [12,196], modulate GST activity [196], and are antiinflammatory [19].

Alcohol, on the other hand, may have detrimental effects. For example, it may enhance penetration of carcinogens by functioning as a solvent; be metabolized to reactive metabolites such as acetaldehyde; produce prostaglandins, lipid peroxidation, and free-radical oxygen species; and/or alter folate metabolism [20,197,198].

Animal studies. Very few studies have examined the effect of coffee on colon cancer in animal models. Mori and Hirono [199] examined the effect of coffee in rats treated with cycasin, a compound derived from the cycad sago palm that is metabolized to the colon carcinogen methylazoxymethanol. In their study, neither coffee nor cycasin alone induced a significant number of tumors. However, coffee and cycasin combined resulted in a high incidence of tumors, indicating that coffee promoted the carcinogenicity of cycasin. Recently, the influence of organic and conventional coffees, each at three different dietary levels (5, 10, and 20%), as well as 4% powdered coffee (eight coffee groups in all), was examined in carcinogen-treated rats [200]. The authors reported no significant effect of the coffee on ACF number. However, it should be noted that every coffee group had a greater number of ACF than the coffee-free control group, in most cases twice as many, raising the question as to whether further statistical analysis might have led to a different conclusion. In contrast, in a study in which 1% coffee was fed to carcinogen-treated rats, no difference in ACF number was found in the coffee group compared to the control group [201]. Since caffeine alone has been shown to increase tumor number and decrease long-term survival in rats treated with an HAA [202], it may be that the caffeine in coffee promotes carcinogenesis, but that phytochemicals within coffee counteract this effect when the quantity of caffeine is low.

Considerably more attention has been focused on the potential chemopreventive effects of tea, both green and black. In a study using an HAA as the carcinogen, green tea, but not black tea, was found to reduce ACF in rats [203]. However, in rats treated with azoxymethane as a carcinogen, the group fed black tea, but not green tea, had fewer adenomas [204]. There were also fewer cancers in the black tea group, but this reduction was not statistically significant. A second study using the colon-specific carcinogen azoxymethane reported that green tea did not reduce the number of ACF [205]. Further, Weisburger et al. [206] reported that extracts of black or green tea given to azoxymethane-treated rats had no influence on tumor development. In contrast, in mice treated with azoxymethane and fed a high fat background diet, mice given 0.6% green tea (0.6 mg tea solids/mL) had significantly fewer ACF relative to mice given only water [207]. White tea, which is the least processed type of tea, and therefore has the greatest quantity of the putative chemopreventive catechins, was found to greatly reduce ACF in rats administered an HAA as the carcinogen [208]. Yet, in a second study by the same investigators, white tea was found to *promote* the formation of colon tumors in rats administered an HAA [202]. Clearly, the results from animal studies on tea and colon carcinogenesis are highly inconsistent, and no conclusions can yet be drawn as to whether tea, in any form, is chemopreventive.

Animal studies examining the effect of fermented beverages such as beer and wine are very few. Feeding beer to carcinogen-treated rats led to a significant reduction in gastrointestinal tumor incidence, but not colon tumor incidence [209]. This finding is consistent with two studies in

which colonic tumor incidence was unaltered by beer consumption, although it led to a shift in tumor incidence from the right and transverse colon to the left colon [210,211]. However, in a more recent study, feeding freeze-dried beer, which contains no ethanol, nor the volatile components of beer, reduced ACF formation when fed in both the initiation and promotion phases of carcinogenesis [212]. When the freeze-dried beer was fed only in the promotion phase, the effect was somewhat attenuated. Ethanol alone had no effect on ACF formation. In contrast to the situation with beer, in which there are studies of feeding the beverage itself, there appear to be no studies in which wine was fed to carcinogen-treated animals. Studies with extracts from wine have been inconsistent. In one study, feeding an extract of complex polyphenols and tannins from wine did not reduce the number of ACF in carcinogen-treated rats [213]. Interestingly, in two subsequent studies by the same investigators, a polyphenolic extract of red wine reduced adenoma incidence in carcinogen-treated rats [214,215]. Given that the polyphenolic extract differs from wine itself, and that the results with the extracts were inconsistent, no conclusion can be drawn regarding the influence of wine consumption on colon carcinogenicity from animal studies.

Human studies. Historically, evidence for a protective association of coffee intake against colon cancer has been somewhat inconsistent. However, there is greater consistency in more recent reports from meta-analyses of prospective cohort and case−control studies, and have mostly been consistent in showing inverse associations between coffee intake and colon cancer risk [216−219]. In particular, the meta-analysis by Gan et al. [218] based solely on prospective cohort studies (19 studies with >22,600 cases combined), showed a threshold ≥5 cups/day as associated with decreased colon cancer risk, which is similar to the earlier suggestion of a nonlinear relationship and report by Tian et al. [220] that ≥4 cups/day is associated with decreased risk of colon cancer. Gan et al. posit that a nonlinear relationship is biologically plausible [218] given coffee's complex mixture of a thousand compounds, some of which may be detrimental (heterocyclic amines, aromatic hydrocarbons, acrylamide, caffeine) as well as the many bioactive compounds discussed earlier. Nonetheless, challenges persist in clearly determining the coffee and colon cancer relationship. For example, subgroups of populations may be more responsive to the effects of coffee, and insufficient measurement of the method of coffee preparation or type of coffee. Instant, filtered, and percolated coffee have negligible amounts of the bioactives cafestol and kahweol (paper filters significantly trap cafestol and kahweol) [221,222]; espresso has intermediate amounts; and Turkish, cafetière, and Scandinavian-type boiled coffees have large amounts [223]. Of note, Gan et al. detected stronger associations in older studies and put forth the notion that coffee preparation methods may have changed over time [218].

The majority of studies on tea have primarily focused on green tea, and frequently indicated a protective effect, but an assessment of the evidence was deemed limited and inconclusive in the WCRF/AICR 2007 report [20]; there have been no further updates on tea and colon cancer risk by WCRF/AICR. However, studies since 2007 seem to consistently report a protective association. Examples include an inverse relationship reported between green tea intake and colon cancer in a cohort of Chinese women [224]. Also, in a randomized control trial of 136 colorectal adenoma patients, adenomas were removed and patients randomized to 1.5 g green tea extract per day or no supplement for 1 year; there were fewer patients with metachronous adenomas in the supplement group ($p < 0.05$) and the size of relapsed adenomas was smaller in patients in the supplement group compared to the control group ($p < 0.001$) [225]. Conversely, in a Singapore cohort there was suggestion of an actual increased risk with green tea for advanced colon cancer in men and no association with black tea [226]. Using urinary biomarkers of tea polyphenols, Yuan et al. observed that in comparing the highest tertile of urinary epigallocatechin to undetected epigallocatechin there was an inverse association with colon cancer [227]; there was a similar inverse relation seen for 4′-*O*-methyl-epigallocatechin, and the strongest protective effect was observed for regular tea drinkers with high levels of both urinary polyphenols [227]. Lastly, in a cohort of Chinese men, green tea intake was associated with reduced risk of colon cancer in male nonsmokers [228]. However, a meta-analysis by Wang et al. [229] based on prospective cohort studies of green tea showed an inverse association with colon cancer only in the Shanghai population. A subsequent meta-analysis by Zhang et al. [230] of prospective studies of all kinds of tea showed no association between the green tea subtype and colon cancer risk.

Alcohol is one of a few dietary exposures with some of the most convincing human evidence for increasing risk of colon cancer [49]. For instance, results of a meta-analysis that included 16 cohort studies indicated that high intake of alcohol increased risk of colon cancer that was equivalent to a 15% increased risk of colon cancer for an increase of 100 g of alcohol per week [231]. A subsequent meta-analysis sought to clarify the dose-risk relation of alcohol to colorectal cancer and found a positive association with >1 drink per day, and the association of alcohol drinking with colorectal cancer did not differ by colon and rectal subsites [232]. A recent meta-analysis that utilized 12 case−control and 9 cohort studies that assessed the risk related specifically to beer intake, reported increased risk for colorectal cancer that

was stronger for rectal (30% increased risk) than colon cancer (5% increased risk) [233].

VII SUMMARY

With regard to fruits, vegetables, and legumes intake, there is some suggestion from animal studies of protection against colon cancer by fruit and soy, but not other legumes. The animal-based evidence for protective effects from vegetables is stronger. Evidence from human studies is inconsistent. While animal data are generally not supportive of meat increasing colon cancer risk, there may be an important interaction with dietary calcium, such that meat may promote colon cancer in the context of a low calcium diet. Human studies are more consistent with regard to meat consumption increasing colon cancer risk, although a clear mechanism is lacking. Strong data from animal studies are supportive of a protective effect from calcium, but the animal data is inconsistent for vitamin D and virtually absent for milk and dairy. There is some consistency in the human data regarding milk and dairy intake, and possibly calcium, but not regarding vitamin D. Relatively few studies with whole grains have been conducted in animals. Those examining wheat suggest that wheat color, not refining state (i.e., whole vs refined), is the important factor for reducing colon cancer risk. Studies using only wheat bran are supportive of a chemopreventive potential. There is little support from animal studies for other cereals being protective against colon cancer. Evidence in humans is limited on whole grains, but is generally encouraging of potential protection. Finally, with regards to nonnutritive beverages, few animal studies have been done on coffee and the tea and alcohol data are inconsistent. Likewise, human data are inconsistent for coffee and somewhat inconsistent for tea, but strong or convincing for alcohol increasing risk of colon cancer.

While this chapter presented the current state of the evidence for associations between food groups and colon cancer, it cannot be overlooked that foods are rarely eaten in isolation; they are consumed as part of a larger, complex dietary pattern. Interest and methodological developments are improving for assessment of the associations of different overall dietary patterns with colon cancer risk. For instance, a study in the United States (North Carolina) investigating risk modification of colon cancer identified three distinct dietary patterns and compared their associations with colon cancer [234]. The three dietary patterns were "Western-Southern" (high in red meats, fried foods, cheese dishes, sweets), "fruit-vegetable" (high in fruits, vegetables, legumes), and "metropolitan" (salad, seafood, pastas, Mexican foods, turkey, chicken, veal, lamb, cruciferous vegetables, alfalfa sprouts). The "fruit-vegetable" pattern was significantly inversely associated with colon cancer risk in Whites but not in African-Americans. A Canadian case–control study assessed three patterns: Meat-diet pattern (high intake of red and processed meat), Sugary-diet pattern (high intake of fruit pies, tarts, desserts, and sweets), and Plant-based pattern (fruits, vegetables, whole grains); increased risk of colon cancer was observed for the Sugary-diet pattern (proximal and distal) and the Meat-diet pattern (distal) [235]. In a large cohort of women in the United States, a diet pattern characterized by higher meat, fish, and sweetened beverage intake but lower coffee, high fat dairy, and whole-grain intake was associated with colon cancer in those who were overweight or sedentary [236]. For comparison, it was observed in a Japanese cohort that a high dairy, high fruit and vegetable, low alcohol dietary pattern was inversely associated with colorectal cancer, but only rectal cancer upon analyses by subsite; no associations were observed for a Japanese dietary pattern or an "animal food" pattern [237]. Lastly, a meta-analysis that included eight cohort studies and eight case–control studies found an increased risk of colon cancer associated with a "western" diet of high red and processed meat intake, and a decreased risk associated with a "healthy" pattern of high fruit and vegetable intake [238].

There is a general paucity of whole food studies in the body of literature on nutrition and colon cancer, which represents a severe limitation in diet and cancer research. People eat food as opposed to individual constituents or fractions and a presumption that there are no differences between pure individual constituents and intact foods is clearly false in some cases [57]. A greater use of foods in animal and human studies is needed to get us closer to developing appropriate dietary recommendations to make regarding cancer prevention. Additionally, there are relatively few whole food feeding intervention trials in humans. While of necessity they rely on intermediary markers of colon cancer, they could prove valuable in testing or confirming hypotheses and findings from population-based studies and animal studies [239]. Furthermore, future human studies may need to better account for genetic variation among individuals which can not only impact metabolism of carcinogens (as briefly mentioned earlier) but may impact tolerance, absorption, and metabolism of the putative chemopreventive constituents in the diet [240].

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Chapter 37

Intestinal Microbiota and Diet in Health

Merlin W. Ariefdjohan, Abby Dilk, Onikia N. Brown-Esters and Dennis A. Savaiano
Purdue University, West Lafayette, IN, United States

I INTRODUCTION

The human gastrointestinal (GI) tract contains bacterial communities that are diverse and complex. This intestinal microbiota plays an important role in digestion and production of essential vitamins and protects the GI tract from pathogen colonization. Although the intestinal microbiota appears to be relatively stable, it can be altered by environmental factors such as disease, antibiotics, and diet. Furthermore, it has been postulated that dominant bacterial groups influence the well-being of their host by forming close interactions with the mammalian cells.

Over the past decade, there has been substantial progress in understanding the GI microbe−host relationship, primarily because of methodological advancements. Consequently, the mechanisms by which the intestinal microbiota influences human health and disease are becoming better understood. Scientific breakthroughs in this field have permitted researchers to move from observation to the prediction of disease using biomarkers based on the metabolic capabilities of intestinal microbiota. Dietary intervention strategies including the consumption of prebiotics, probiotics, and synbiotics have also been developed to enhance overall health and reduce disease incidence. Therefore, understanding the intricate relationship between GI tract microbiota and health is important for improving the quality of life for many.

This chapter serves to provide a comprehensive review of the following: (1) the concept of intestinal microbiota, (2) the methodology used to investigate the intestinal microbiota and their limitations, (3) the influence of diet on intestinal microbiota, and (4) future directions in the field.

II DISTRIBUTION AND DIVERSITY OF THE HUMAN INTESTINAL MICROBIOTA

When the word *ecosystem* comes to mind, most people conjure up images of the rain forest with its myriads of animals, plants, and insects or the oceans teeming with fish, algae, and phytoplankton. Rarely do we think of the ecosystem in our body in the form of bacterial communities residing on the skin, oral cavity, genitals, and GI tract. On average, the human GI tract (i.e., small intestine and large intestine [colon]) is 27 ft long. Its surface area is greatly enhanced by the formation of microvilli on the surface of each intestinal villus (Fig. 37.1). Because of this anatomical folding, the GI tract creates a large surface area for bacterial colonization (approximately 150−200 m^2 or 1614−2153 ft^2) [1]. As a comparison, the human skin covers only approximately 2 m^2 (21.5 ft^2). The adult GI tract is estimated to harbor up to 10^{14} bacteria/g of intestinal contents. This number easily exceeds the population size of other microbial communities associated with the human body. Remarkably, the microbial population in the GI tract is approximately 10 times greater than the total number of cells in the human body [2].

The intestinal microbiota of the adult GI tract is composed of all three domains of life—Bacteria, Archaea, and Eukarya, with bacteria having the highest cell densities [3]. The flora is distributed along the entire GI tract from the esophagus to the rectum (Fig. 37.2). The intestinal microbiota is also distributed in a vertical gradient within a specific part of the GI tract. Four microhabitats have been described (Fig. 37.3): the intestinal lumen, the unstirred water layer, the mucous layer at the surface of the mucosal epithelial cells, and the mucus layer in the intestinal crypts [4].

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The population size and diversity of the intestinal microbiota are influenced by intrinsic factors such as pH, secretion of intestinal fluids, and transit time. The stomach and duodenum create a harsh environment for bacterial colonization. The former has low pH (ranging from 2.5 to 3.5) due to secretions of intestinal fluids (e.g., hydrochloric acid), and the latter has a short transit time. Consequently, approximately only 10^1-10^3 bacteria/mL of intestinal content reside in the stomach and duodenum, with a majority of them being transient. As the environment becomes less acidic and transit time gradually increases toward the distal end of the GI tract, the microbiota community flourishes both in number and in diversity (Fig. 37.2).

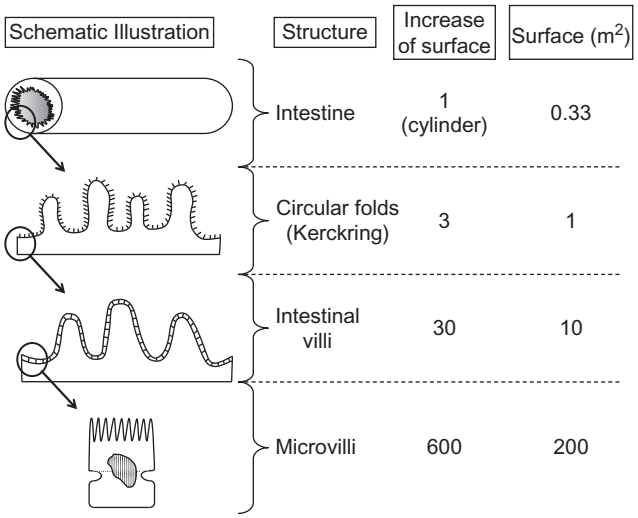

FIGURE 37.1 Folding on the intestinal mucosa significantly increased the surface area of the GI tract, providing a large surface area for bacterial colonization. *Modified from F. Waldeck, Funktionen des magen-darm-kanals, in: R. Schmidt, G. Thews (Eds.), Physiologie des Menschen, Springer, Berlin, 1990, p. 24.*

In comparison to the other parts of the GI tract, the colon has the slowest cell turnover rate, the lowest redox potential, and the longest transit time. Hence, the colon harbors the most diverse and the highest number of bacteria (Fig. 37.2). The colon is the major site for bacterial fermentation of nondigestible food components. Approximately $10^{10}-10^{12}$ bacteria/mL of intestinal contents reside in the colon. This microbial population includes more than 500 species belonging to more than 190 genera [5]. Because of the complexity of collecting samples from within the GI tract, most studies investigating the intestinal microbiota have been based on fecal sample analyses. Thus, our current understanding of the population size may not accurately reflect "true" species abundance and their relative importance in metabolic processes [6].

A few major groups of strict anaerobes dominate the colonic microbiota community, including *Bacteroides* spp., *Eubacterium* spp., and *Bifidobacterium* spp. [7]. Facultative aerobes such as *Enterobacter* spp., *Streptococcus* spp., and *Lactobacillus* spp. are also present as subdominant flora. Minor groups of pathogenic and opportunistic microbiota (e.g., *Clostridium* spp. and *Vibrio* spp.) are also present in low numbers [8]. The metabolic functions of many of the dominant bacterial species in the GI tract are not well understood. However, several studies indicate that *Bifidobacterium* spp. and *Lactobacillus* spp. are intestinal bacterial species that directly contribute to health [9] (Fig. 37.4).

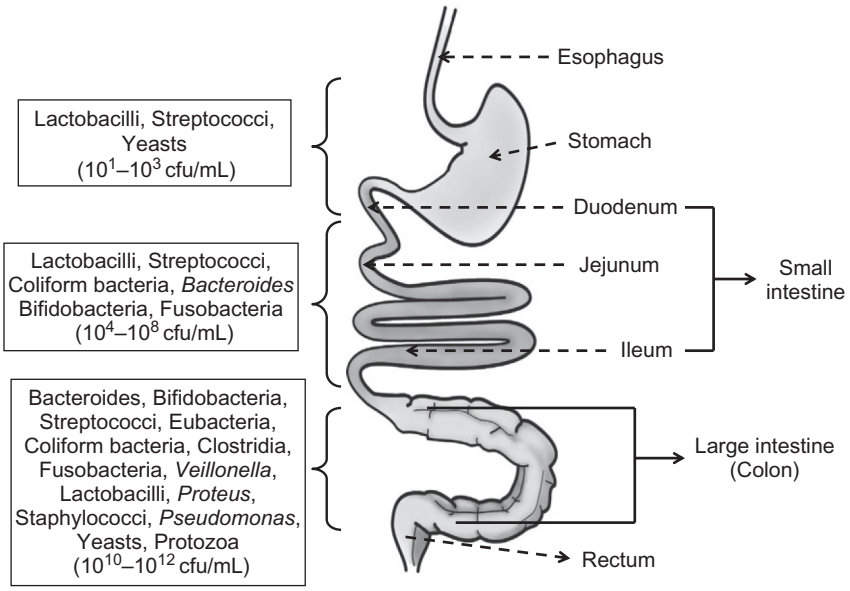

FIGURE 37.2 The human GI tract and the distribution of the intestinal microbiota. *Modified from G. Simon, S. Gorbach, Intestinal microbiota, Med. Clin. North Am. 66 (1982) 557–574.*

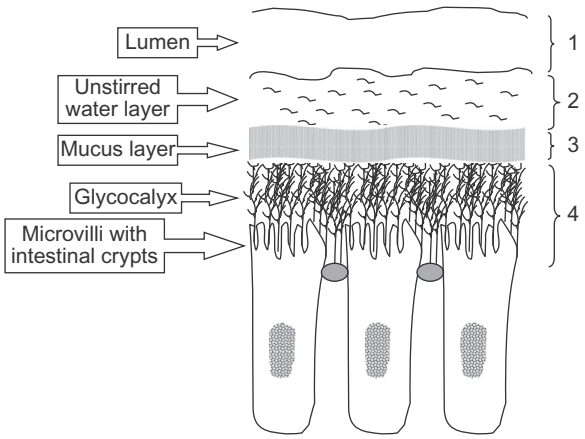

FIGURE 37.3 The four microhabitats within the GI tract representing the vertical distribution of the intestinal microbiota: (1) the intestinal lumen, (2) the unstirred water layer, (3) the mucus layer at the surface of the mucosal epithelial cells, and (4) the mucus layer in the intestinal crypts.

III BACTERIAL COLONIZATION, SUCCESSION, AND METABOLISM

A Colonization and Succession

The GI tract of a fetus is believed to be sterile [10,11]. Bacterial colonization occurs immediately at delivery and gradually becomes more extensive as the newborn is introduced to the living environment and various foods [6,10–14]. Previous theories suggested that bacterial colonization of a breast-fed infant's gut was solely the result of contamination to the infant's oral cavity or from the mother's skin [15]. However, recent studies indicate that *Lactobacillus* spp. and *Bifidobacterium* spp. can translocate from the maternal GI tract, endogenously become incorporated into the mammary glands, and then reach the fetal GI tract where the bacteria can colonize. Furthermore, human milk oligosaccharides (HMOs) have been shown to enhance the growth of certain bacteria,

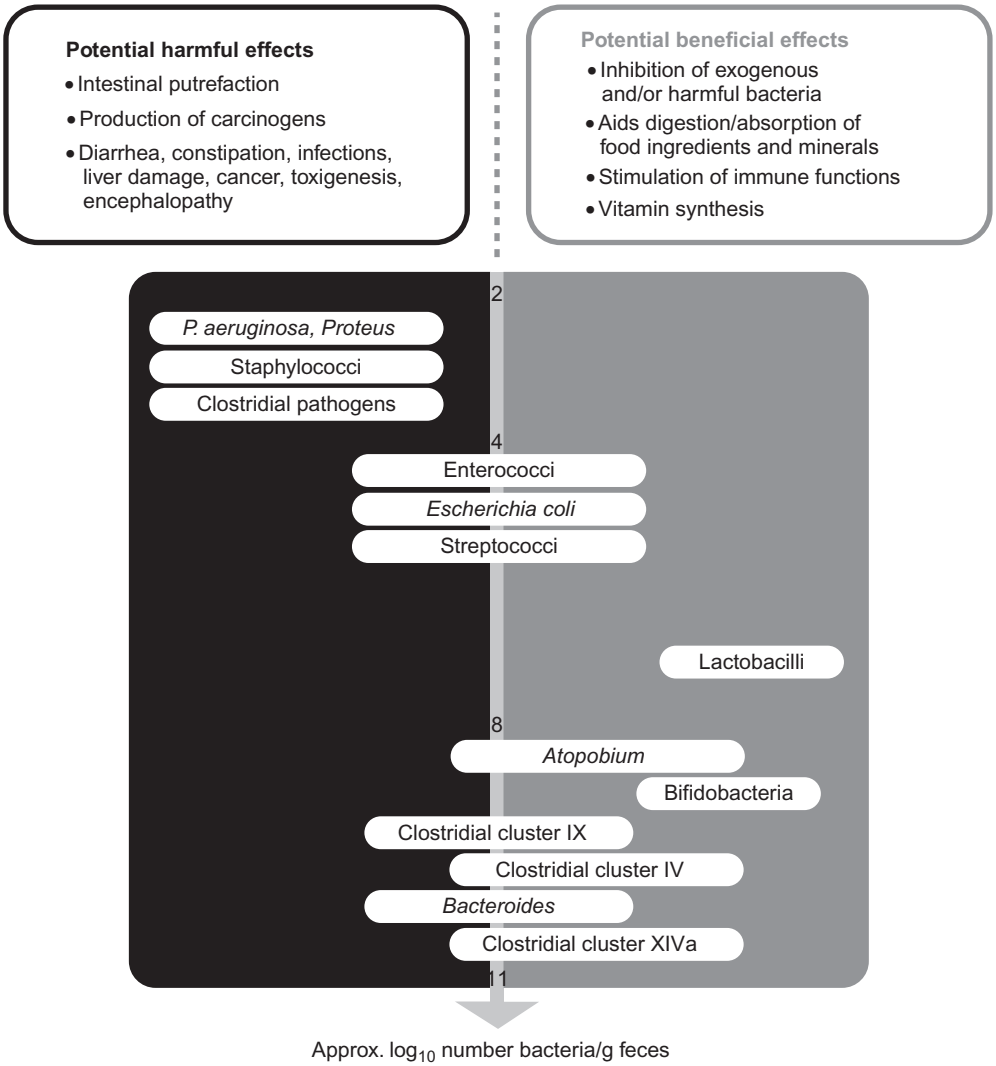

FIGURE 37.4 Dominant intestinal microbiota as categorized into potentially harmful or health-promoting groups. *Reprinted with permission from L. Thomas, M. Flower, A guide for healthcare professionals:* Lactobacillus casei *Shirota and Yakult, Science for Health, Yakult, UK, 2006.*

including *Bifidobacterium longum bioar infantis* [16]. HMOs also block pathogen adhesion, which can protect breast-fed infants from common infections, diarrhea, and even HIV transmission from the mother. This obstruction results from HMOs structural similarity to intestinal epithelial cell surface glycans that pathogens must attach to before invading the host. HMOs have also been shown to induce apoptosis and differentiation of intestinal epithelial cells. Bacterial colonization of the GI tract occurs in four phases [17,18]. Phase I, the initial acquisition phase, occurs between birth and 1 or 2 weeks. Phase II is considered a transitional period that takes place during lactation with either breast milk or formula. This event typically starts at the end of the second week after-birth and ends when supplementary feeding begins. Phase III is initiated with the introduction of other food sources, especially at weaning (e.g., when breast milk or formula is supplemented with solid foods). Phase IV follows once weaning is completed and the child is introduced to an adult diet.

In phase I, *Enterobacter* and *Streptococcus* appear to colonize the infant GI tract within 48 hours of birth. *Escherichia coli* follows soon after. These bacteria are thought to create a favorable environment for subsequent colonization by anaerobic bacteria including *Bacteroides* spp., *Bifidobacterium* spp., and *Clostridium* spp. This secondary colonization usually occurs within 4–7 days [6,19,20]. During later stages of phase I, a marked reduction in the levels of *E. coli* and *Streptococcus* was observed in the stools of exclusively breast-fed infants. This event was followed by a decrease in the levels of *Clostridium* and *Bacteroides* spp., whereas *Bifidobacterium* spp. gradually dominate. It appears that these trends are not as distinct in formula-fed infants [17]. Breast-fed infant GI microbiota appears to be more stable and predominantly populated by *Bifidobacterium* and *Bacteroides* spp., whereas formula-fed infant microbiota is more diverse but less stable, containing less *Bifidobacterium*, *Lactobacillus*, and *Staphylococcus* spp. but more *Bacteroides*, *Enterococcus*, and *Streptococcus* spp. [21]. The intestinal microbiota of formula-fed neonates also harbors members of the Clostridia class and Enterobacteriacae family, which are potential pathogens. However, the lesser diversity seen in breast-fed infants' intestinal microbiota is disadvantageous and may be associated with several diseases.

In phase II, *Bifidobacterium* spp. remain at high levels and become the dominant bacterial group in the intestinal microbiota of breast-fed infants [17]. Apparently, this is different from the intestinal microbiota profile of formula-fed infants, in whom relatively high numbers of *Bacteroides* spp., *Clostridium* spp., and *Streptococcus* spp. are present and *Bifidobacterium* spp. is no longer the dominant group [17].

The introduction of new foods to infants in phase III results in a major shift in microbial succession [12,13,17,20]. This shift is more significant in breast-fed infants. Following weaning, the intestinal microbiota of breast-fed infants gradually changes to resemble the community found in formula-fed infants. *Clostridium* spp., *Streptococcus* spp. and *E. coli* reappear, followed by *Bacteroides* spp. and other anaerobic gram-positive cocci such as *Peptococcus* spp. and *Peptostreptococcus* spp. [20]. At the end of phase III, differences in the intestinal microbiota of breast-fed and formula-fed infants are no longer observed.

Bacterial succession continues until weaning is completed (i.e., beginning of phase IV). This phase is denoted by a continued increase in *Bacteroides* spp. and anaerobic gram-positive cocci. Colonic levels of *Bifidobacterium* spp. continue to remain high. At this time, *Bifidobacterium* spp. is found in all individuals regardless of their starting diet [17]. *Escherichia coli* and *Streptococcus* spp. gradually decline to a typical adult level (i.e., approximately 10^6–10^8 bacteria/g of feces) [22]. *Clostridium* spp. is also present [22]. At the end of phase IV, the infant intestinal microbiota begins to resemble the bacterial community profile of an adult, which is typically diverse but relatively stable [12,13,18] (Fig. 37.5). Phase IV is usually attained by 2 years of age [12,13,20].

During adulthood, the intestinal microbiota can be modified by external factors including diet, environment, and medication. Due to reasons that are still unclear, the number of bacterial species residing in the GI tract declines with age [23–25] (Fig. 37.5). Typically, the population levels (in terms of $\log_{10}$ CFU1g) of *Bifidobacterium* spp. are markedly decreased and reduced to one or two dominant species, particularly *B. adolescentis* and *B. longum* [23,26]. In contrast, *Enterococcus* spp., *Lactobacillus* spp., *Bacteroides* spp., and *Clostridium* spp. are increased in GI tracts of the elderly [23,25].

B Metabolic Consequences of Bacterial Colonization and Succession

Changes in the intestinal microbiota of infants due to bacterial colonization and succession are reflected metabolically, especially in terms of fecal short-chain fatty acid (SCFA) profiles [27]. During the period when *Bifidobacterium* spp. predominate in the GI tract of breast-fed infants, acetic acid is the major SCFA detected [28,29]. Accumulation of acetic acid decreases the stool pH from values originally observed at birth. This trend is expected because *Bifidobacterium* spp. are a major producer of acetic acid. In formula-fed infants, however, lower levels of acetic acid are present and the stool pH tends to rise slightly. Therefore, changes in the fecal SCFA profile of breast-fed infants appear to be more profound than those in formula-fed infants [17,29]. Breast-

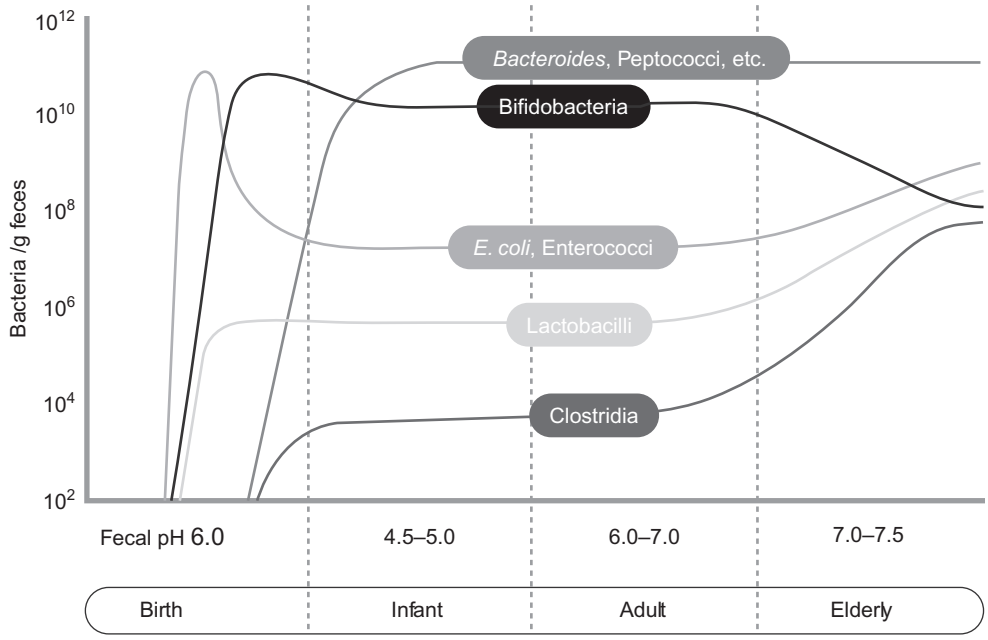

FIGURE 37.5 Bacterial succession throughout the lifetime. *Reprinted with permission from L. Thomas, M. Flower, A guide for healthcare professionals:* Lactobacillus casei *Shirota and Yakult, Science for Health, Yakult, UK, 2006.*

fed infants are also observed to exhibit a gradual increase in total SCFA. Concurrently, lactic acid decreases while acetic and propionic acid increase. At later stages of weaning, butyric acid production gradually increases as well. In contrast, changes in the fecal SCFA profile of formula-fed infants are less profound [17,29,30]. This may be attributed to fewer fluctuations in the GI microbiota in these infants.

The ability to ferment complex carbohydrates from diet may also reflect the variability in bacterial succession rate in breast-fed and formula-fed infants. Although variations exist among individual infants, fermentation capability appears to develop faster in formula-fed infants than in breast-fed infants [30]. This is expected because the colons of formula-fed infants are inhabited by more diverse bacterial strains and a greater population of gram-negative anaerobes, many of which are involved in the fermentation of complex carbohydrates. Unlike breast-fed infants, the colonic fermentation capacity of formula-fed infants does not vary significantly through weaning stages [30]. This observation suggests that the colonic microbiota of formula-fed infants matures faster than that of breast-fed infants and does not experience major shifts in composition [29–31].

C Factors Influencing Bacterial Colonization and Succession

In phase I of colonization, environmental factors introduce bacteria to the infant GI tract. With infants born by vaginal delivery, the length of the birthing process significantly influences the chance of detecting viable bacteria from the mouth and stomach of the newborn [32]. Infants born by cesarean section are exposed to and may acquire the mother's microbiota. However, initial exposure is most likely environmental (e.g., hospital condition, nursing staff, and other infants in the nursery) [33–36]. Cesarean sections may be associated with a 46% higher offspring risk of childhood obesity [37]. Furthermore, antibiotics used by the mother during the second or third trimester may be associated with a higher offspring risk of childhood obesity. Thus, these findings provide evidence that the gut microbiome influences health status later in life. In addition to mode of delivery, hygiene practices may influence the bacterial species introduced to the infant GI tract. For example, bacterial colonization of the GI tract of Pakistani infants born in poor areas with minimal sanitation occurred significantly earlier than in Swedish infants delivered in more sanitary conditions, regardless of delivery method [14].

There are numerous external and internal factors that shape the composition of the microbial community in the GI tract (Fig. 37.6). External factors include mode of birth, composition of the diet, sanitation of the living environment, and the use of medicines, especially antibiotics. Internal factors include changes in the physiological condition of the host (e.g., stress, health status, and aging) and conditions in the GI tract (e.g., pH, substrate availability, redox potential, transit time, flow of enteric fluid, and IgA secretions) [6]. A disruption of the "normal" intestinal bacteria may lead to the growth of those that are potentially harmful and physiologically manifest as a disease. Studies show that changes in diet, climate, aging, medication, illness, stress, and/or infection generally lead

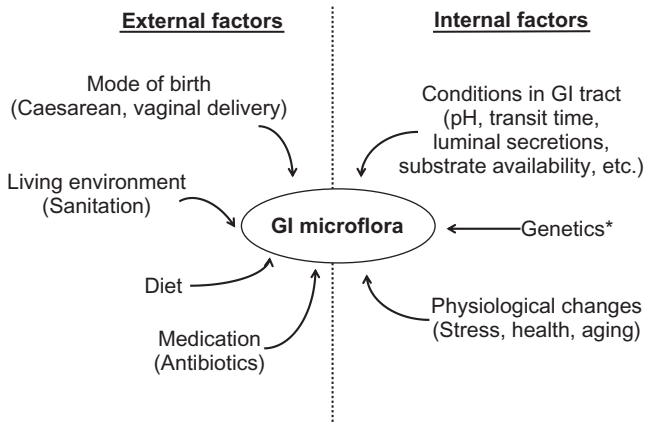

FIGURE 37.6 External and internal factors influencing bacterial succession in the GI tract. *Genetics has been speculated to play a role in intestinal microbiota succession, but the current body of literature has not provided strong evidence for it.

to an increase in anaerobes and *E. coli* in the small intestine and to an increase of *Enterobacter* and streptococci in the colon, with a concurrent decrease of *Bifidobacterium* spp. [37,38]. Although predicting the occurrence of a disease based on intestinal microbiota profile is currently not possible, it is hypothesized that a marked decline in intestinal bacteria that are beneficial to health, such as *Bifidobacterium* spp., will ultimately influence the health of the host.

IV FUNCTIONS OF THE GI TRACT MICROBIOTA

A Production of Vitamin K

Prothrombin, which is involved in blood clotting, is activated by vitamin K. Food sources of vitamin K are liver, eggs, milk, and spinach. However, this vitamin can also be synthesized by bacteria in the GI tract. Administration of antibiotics eradicates intestinal bacteria and consequently may diminish de novo vitamin K production. Newborn infants, whose GI tracts are typically devoid of bacteria, may be given an injection of vitamin K at birth to counter deficiency until their vitamin K-producing bacteria are established [39]. Another vitamin that is exclusively synthesized by bacteria is vitamin B_{12} (cobalamin) [40], which is important for the formation of red blood cells.

B Protection Against Pathogens

Colonization of the GI tract by the intestinal microbiota confers protection on the human host. A fully established GI tract microbiota community can "outcompete" pathogens for carbon and other energy sources, as well as for adhesion sites on the intestinal mucosa. Consequently, pathogens are less able to establish themselves on the intestinal mucosa and thus are prevented from causing physiological damage to the host [41]. Complex microbiomes may result in high expression of RegIIIγ by epithelial cells, which is a C-type lectin that restricts pathogen-epithelium contact [42]. This effect is likely dependent on IgA due to its ability to prevent bacterial adhesion and epithelial stimulation. Some species of indigenous intestinal microbiota may also produce bacteriocins that kill pathogens [43].

C Enhanced Histological and Physiological Development of the GI Tract and the Immune System

Intestinal microbiota plays a role in the development of intestinal mucosa and gut-associated lymphoid tissue (GALT). Evidence for this role was provided by comparative assessments of histological and physiological data from germ-free (GF) mice and conventionally raised (CONV-R) mice. GF mice are devoid of intestinal microbiota because they are delivered by cesarean and raised in a sterile environment. On the other hand, CONV-R mice are delivered and raised in a standard environment in order to acquire a "normal" intestinal microbiota. Histological data show that CONV-R mice have a higher epithelial cell turnover rate than that of GF mice (i.e., 2 days in CONV-R vs 4 days in GF). In addition, the secondary lymphatic organs of GF mice (e.g., GALT and spleen) are significantly less developed than those of CONV-R mice [3]. Other morphological abnormalities seen in GF mice include an enlarged cecum, larger enterochromaffin cell area, reduced intestinal surface area, and small villi thickness [44]. The intestinal microbiota has also been found to stimulate the development of immune tissues in the GI tract such as the lamina propria, Peyer's patches, and mesenteric lymph nodes. In the absence of the intestinal microbiota, these tissues do not fully develop and become less "primed" to fight infection. The serum of GF mice contains lower concentrations of immunoglobulins than that of CONV-R mice; GF mice have a reduced immune function, are more susceptible to severe infection, and usually have poorer survival.

In humans, differences in the composition of intestinal microbiota have also been suggested to influence immune function. Interleukin-22, Reg3γ, IgA, and interleukin-17 immune responses are likely influenced by the microbiome's production of polysaccharide A, SCFAs, and α-galactosylceramide and tryptophan metabolites [42]. *Bacteroides fragilis* can produce polysaccharide A in outer membrane vesicles, which can then be detected by dendritic cells and prevent inflammation of the colon [45]. *Bacteroides fragilis* also releases α-galactosylceramide, which is a glycosphingolipid that can bind with CD1d

proteins and subsequently activate invariant natural killer T cells. Furthermore, GI microbiota have been shown to impact lamina propria dendritic cells and whether their response will be inflammatory or antiinflammatory to specific antigens [42]. Evidence for this is derived from comparing the prevalence of allergies and atopic diseases in infants [46]. GI tracts of infants born and raised in developing countries (i.e., assumed to have a low level of sanitation) appear to be colonized at an early stage by gram-negative bacteria and variable enterobacterial strains. On the other hand, infants in developed countries (i.e., assumed to have a high level of sanitation) acquired gram-negative bacteria later and more stable enterobacterial strains. Such differences in the intestinal microbiota composition have been associated with a higher prevalence of allergies and atopic diseases in infants born and raised in developed countries than those in developing countries [46]. A study that compared the intestinal microbiota profiles of children in Europe observed that Swedish and Estonian toddlers with low counts of *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides* spp., and *Enterococcus* spp., but having higher levels of clostridia and *Staphylococcus aureus*, were more prone to allergies than were healthy infants [47,48]. Much remains to be learned about intestinal colonization, immune function, and disease in humans.

D Production of SCFAs

Food components that are not digested in the small intestine travel to the colon. These nondigestible elements are substrates for fermentation by the intestinal microbiota. By-products of fermentation include carbon dioxide, hydrogen, and methane gases. Nongaseous by-products include SCFAs such as acetate, propionate, and butyrate (Fig. 37.7). Butyrate is mainly produced by *Clostridium* spp. and *Eubacterium* spp. [49]. *Roseburia intestinalis*, *Eubacterium rectale*, and *Faecalibacterium prausnitzii* have also been identified as butyrate producers [50,51]. Acetate is produced by *Lactobacillus* spp. and *Bifidobacterium* spp. [49].

Approximately 40–50% of the available energy from carbohydrate in the diet is converted into SCFAs by the colonic microbiota [52]. These volatile fatty acids provide energy for cellular maintenance and metabolism by being passively absorbed by the colonic epithelium (butyrate), liver (propionate), and muscle (acetate) [49,53,54]. Studies suggest that butyrate plays an important role in cellular differentiation and proliferation in the colonic mucosa by inducing apoptosis and may confer protection against colitis and colorectal cancer by modulating oncogene expression [55,56]. Production of SCFAs also lowers intestinal pH, which increases the solubility of minerals such as calcium and magnesium [57,58] and consequently enhances absorption [59]. Furthermore, reduction of colonic pH by accumulation of SCFAs has been hypothesized to protect the intestinal mucosa from being colonized by pathogens that are less tolerant of an acidic environment (e.g., *Helicobacter pylori*) [60]. Additional benefits of SCFAs are outlined in Fig. 37.8.

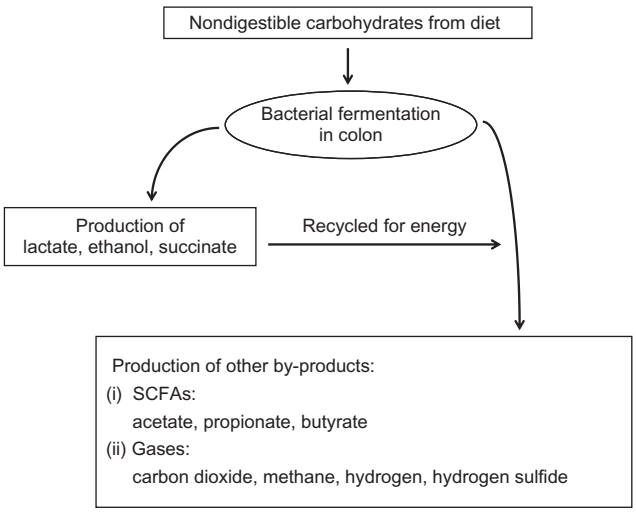

FIGURE 37.7 Bacterial fermentation of nondigestible food components.

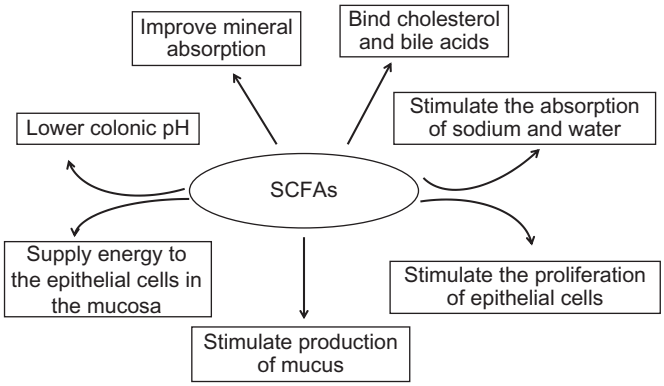

FIGURE 37.8 Benefits of SCFA.

The GI microbiota have been shown to change in composition during pregnancy, which may affect fermentation in the gut and the subsequent production of SCFAs in both peripheral blood and the cecum throughout gestation [61]. Furthermore, fatty acid receptor-2 (Ffar2), a G protein-coupled receptor that binds SCFAs, may be more highly expressed in pancreatic islets during pregnancy. Mice lacking Ffar2 have demonstrated fasting hyperglycemia and impaired glucose tolerance, suggesting that increased SCFA uptake into cells as a result of alterations in the intestinal microbiota contributes to gestational glucose homeostasis.

E Utilization of Nutrients

Absorption of nutrients from food is largely dependent on the action of various digestive enzymes. However, numerous food components cannot be digested. For example, stachyose (a tetrasaccharide) and raffinose (a trisaccharide) are long-chain carbohydrates (i.e., commonly termed oligosaccharides) found in soy. The unique α-(1−6) galactose linkage present in stachyose and raffinose can be broken down by α-galactosidase, which is not secreted by the human intestinal mucosa. As a result, these oligosaccharides (and other nondigestible food components) pass to the large intestine, where they become fermentation substrates for the colonic microbiota. Fructo-oligosaccharides (FOS), transgalacto-oligosaccharides, and galacto-oligosaccharides (GOS) are other types of nondigestible oligosaccharides that can be fermented by the colonic microbiota. Thus, the intestinal microbiota salvages a significant amount of energy from an otherwise nonavailable source [49,52−54].

Evidence that the intestinal microbiota plays an important role in fermenting nondigestible foodstuffs is based on studies using GF and CONV-R mice [3,62]. The latter were more efficient in nutrient absorption than GF mice because they had acquired "normal" intestinal microbiota. CONV-R mice gained more body weight, grew faster, and had as much as 40% more body fat than their GF counterparts even though they were maintained on the same diet. This growth difference was present even though CONV-R mice consumed less chow per day [3]. In order for GF mice to achieve the body weight of the CONV-R counterparts, they had to consume approximately 30% more calories [62]. However, following inoculation of GF mice with the intestinal microbiota of CONV-R mice, GF mice quickly gained body fat to levels equivalent to those of CONV-R mice without having to consume additional chow [62].

GI microbiota also affect fat and micronutrient absorption to such a degree that GF rodents may require a higher caloric intake to maintain proper body weight as compared to mice with colonized GI tracts [63]. Increased pancreatic lipase-related protein-2, colipase, apolipoprotein A-IV, and fatty acid-binding adipose factor, which are all involved in lipid absorption, have been shown to result from microbiome colonization. Furthermore, expression of fasting-induced adipose factor, which is repressed upon fat feeding, is likely to be decreased in GF rats. Additionally, proteins that sequester heavy metals, such as epithelial copper transporter, metallothioneins I and II, and ferritin heavy chain, have been shown to be threefold and five- to sixfold more abundant, respectively, in colonized GI tracts, thereby suggesting that the microbiome may also affect micronutrient absorption.

F Generation of Bioactive Metabolites (Conversion of Isoflavones)

Soy isoflavones, phytochemicals classified as "nonnutritive components," are predominantly derived from soy products. Currently, soy is the only recognized nutritionally relevant source of isoflavones. The primary isoflavones in soy are genistin and daidzin. Following ingestion, these glycosides are hydrolyzed by intestinal glucosidases and converted to the aglycone form of genistein and daidzein. These aglycones are further converted by certain intestinal microbiota into specific metabolites, such as equol. Chemically, equol is similar to the hormone estradiol. Results of in vitro animal studies indicate that equol has a higher estrogenic effect than its precursor, daidzein [64]. Hence, equol has garnered much attention for its potential in the prevention and/or treatment of chronic diseases or conditions associated with estrogen levels (e.g., breast cancer, osteoporosis, and menopause) [65].

In humans, the conversion of soy isoflavones (genistein and daidzein) to the more potent metabolite (equol) appears to be dependent on the composition of the intestinal microbiota. Evidence for this conversion comes from animal and clinical studies. First, all rodents are equol producers, except those that are bred GF. Second, infants fed soy-based formulas do not form a substantial amount of equol for the first 4 months of life, coinciding with intestinal microbiota development. In addition, individuals who are known to be equol producers have significantly lower equol excretion after antibiotic treatment [66]. It appears that the large interindividual variability in the intestinal microbiota composition results in only 30−40% of individuals producing equol after soy consumption [64,67−69]. In 2000, Hur and colleagues [70] identified two strains of bacteria from human feces that can produce primary and secondary metabolites from the natural isoflavone glycosides daidzein and genistin, but it is still unclear whether the ability to convert daidzein to equol can be induced in nonproducers [71].

G Communication With the Nervous System

Gut microbiota and the central nervous system have been found to display bidirectional communication via the microbiota−gut−brain axis [72]. The vagus nerve, humoral components of the immune system, and the hypothalamus-pituitary-adrenal axis are believed to mediate this interaction. Disruption of this axis has been shown to be an underlying cause of irritable bowel syndrome (IBS) and other functional bowel disorders. Furthermore, stress-related psychiatric disorders such as depression and anxiety usually coincide with IBS, which provides additional evidence to support the theory of the microbiota−gut−brain axis. Additionally, changes in gut microbiota have been indicated in autism, and early-life stressful events have been shown to alter brain neurochemistry and the composition of GI microbiota, as well as increase in visceral sensitivity.

A lack of bacterial colonization in the gut during development in GF mice has been linked to higher levels of corticosterone and adrenocorticotrophin levels in response to stress [72]. GF mice were found to have genetic alternations related to the citric acid cycle, steroid hormone metabolism, cAMP signaling, and long-term potentiation [73]. Monoamines such as epinephrine, serotonin, and melatonin have also been shown to exhibit decreased turnover rates as a result of GF GI tracts, as well as decreased production of brain-derived neurotrophic factor, a protein that supports and encourages the growth of neurons and synapses in the brain.

H Promotion of Angiogenesis

Intestinal microbiota colonization likely affects the development and regulation of intestinal angiogenesis via Paneth cells [74]. Bacterial colonization in the intestine and the development of villi blood vessel networks are coinciding after-birth events. Paneth cells are located in intestinal crypts and secrete antimicrobial peptides such as lysozymes and secretory phospholipase A_2, which ultimately affect the ecology of microbes found in the intestinal lumen. Paneth cells can produce factors that promote angiogenesis of the intestine even in the absence of intestinal microbiota; however Paneth cells and microbiota are likely needed in conjunction in order for proper angiogenesis to occur.

V METHODOLOGY FOR STUDYING INTESTINAL MICROBIOTA

A Conventional Methodology and Its Limitations

Our knowledge of intestinal microbiota is largely based on classical approaches of cultivation, direct microscopic observation, and biochemical analysis [75]. Results obtained using these conventional methodologies have improved our understanding of the intestinal microbiota. However, many intestinal bacteria are difficult to culture because the media may not be specific (i.e., causing overestimation) or may be too selective (i.e., resulting in underestimation or absence of growth) for culturing the particular bacteria of interest [76]. It is estimated that only 20−40% [77−79] of the total intestinal microbiota can be cultured and identified using conventional microbiological methods. Thus, evaluating intestinal microbiota using conventional methods will bias our knowledge in favor of those genera that are most easily grown under laboratory conditions [80]. In addition, media preparation and biochemical analyses associated with these approaches are also time-consuming.

B Molecular Analysis of the Human Intestinal Microbiota

In the past two decades, novel analytical approaches based on the manipulation of 16S rDNA and other genetic materials have been developed to analyze bacterial communities in environmental samples (e.g., soil, lakes, oceans, and hydrothermal vents). These methods have been adapted to evaluate bacterial communities in the GI tract [9,81]. Molecular techniques do not require the presence of viable bacteria [9,82,83]. Furthermore, the use of genetic materials (e.g., DNA and RNA) allows species that cannot be cultured using current standard laboratory protocols to be detected. Thus, data derived from molecular techniques depict a more complete and real picture of the bacterial community. These molecular methods have shown a great potential to overcome the limitations associated with conventional techniques and have become increasingly favored [82,83]. Many molecular techniques, especially after being optimized, are now being developed as rapid assays, allowing large-scale studies with high-throughput analyses. Biological samples can now be frozen at the site of collection and then transported to laboratories for analyses. However, because RNA is degraded more easily than DNA, sample storage and handling largely depend on the choice of method and outcome measures.

Molecular techniques can be categorized as follows:

1. Direct molecular detection and/or enumeration: dot blot hybridization, fluorescent in situ hybridization (FISH), and real-time polymerase chain reaction (RT-PCR);
2. Molecular fingerprinting techniques to monitor changes in the composition of bacterial community: terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE);

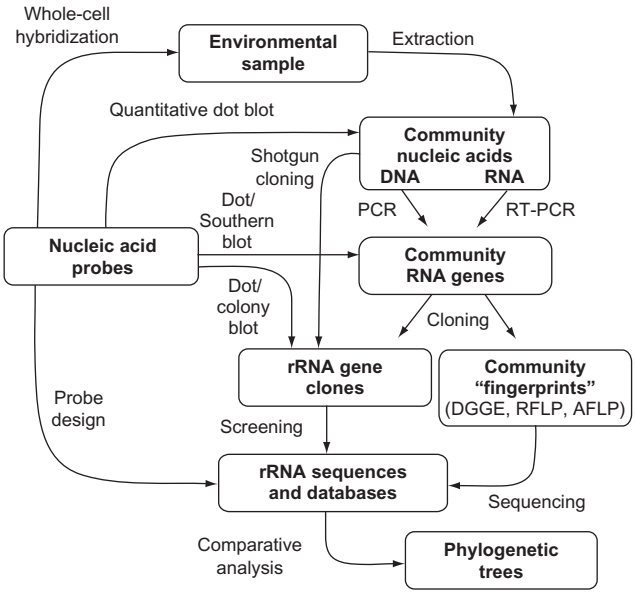

FIGURE 37.9 Various molecular methods and analytical approaches that are applicable for studying the intestinal microbiota communities. *Reprinted with permission from P. Lawson, Taxonomy and systematics of predominant gut anaerobes, in: G.R. Gibson, M.B. Roberfroid, Colonic Microbiota, Nutrition and Health, Kluwer, Dordrecht, the Netherlands, 1999, pp. 149–166.*

3. Genotyping: rapid amplification polymorphic DNA–polymerase chain reaction (RAPD-PCR), enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR), multilocus sequence typing, plasmid profile analysis, repetitive extragenic palindromic, ERIC, ribotyping, as well as amplified fragment length polymorphism (ALFP), RFLP, and RAPD techniques;
4. Other novel molecular methods under development: microarray, magnetic-immuno PCR, and *recA* gene analysis.

Other molecular techniques have been developed to study microbial communities in the environment. Fig. 37.9 outlines several common methods of analysis.

C Limitations Associated With Molecular Techniques

Despite their superiority to conventional microbiological approaches, molecular techniques do have limitations. The main drawback of molecular techniques, which significantly influences downstream analytical processes, is their reliance on cell lysis efficiency and the quality of DNA recovered from the environmental samples. DNA isolation methods that contribute to insufficient cell lysis or shearing of DNA bias PCR amplification [84–87]. Inhibitors in feces, such as bile salts and complex polysaccharides, will create similar problems [84,88,89]. Furthermore, special equipment and reagents are needed for sample processing and analyses (e.g., thermal cycler, RT-PCR unit, DGGE setup, bead-beating equipment for lysing cells, and DNA or RNA extraction kits). Thus, initial laboratory setup may be costly, especially for novice researchers. However, research in this field has advanced rapidly in approximately the past decade. Technological progress has significantly reduced costs as well as improved quality and performance of equipment and reagents. Note that some molecular techniques are semiquantitative (DGGE and TGGE) and may lack sensitivity (e.g., dot blot hybridization and FISH). Primer bias may also occur in PCR reactions. In addition, almost all of these molecular techniques rely on primers and oligonucleotide probes to detect bacteria. Hence, the choice of primers and probes in analyses is crucial for detecting the bacteria of interest. Although primer and probe availability may be limited, their numbers are steadily increasing as interest in this field grows. Despite these limitations, molecular techniques are continually being developed and modified to increase efficiency and sensitivity in analyzing the diverse bacterial community in environmental samples, including those found in the GI tract and feces.

VI INFLUENCE OF DIET ON INTESTINAL MICROBIOTA

Several bacterial genera make up a large majority of the intestinal microbiota (Figs. 37.2 and 37.4). However, external and internal factors can modify the overall number and dominant species within the community (i.e., the concept of microbial succession; Fig. 37.6). One of the most studied external factors that influences microbial succession is diet.

A Can Diet Alter the Intestinal Microbiota?

Comparative analyses of fecal samples from breast-fed and formula-fed infants have shown differences in the composition of their intestinal microbiota as a response to the diet (Figs. 37.10 and 37.11). The intestinal microbiota of adults is more diverse and stable than that of infants; however, it can still be modified by diet. In 1974, Finegold et al. [90] compared the fecal flora of subjects who consumed a traditional Japanese diet to that of those who consumed a Western diet. In this study, a significantly higher number of *Clostridium* spp., *Eubacterium* spp., and *E. coli* were recovered in the feces of subjects on the traditional Japanese diet than in those on the Western diet. On the other hand, *Bacteroides* spp. (especially *B. infantis* and *B. putredinis*) and *Bifidobacterium* spp. were more prevalent in the fecal samples of subjects on the Western diet. In addition, differences in intestinal bacterial profiles were also found to vary by ethnicity (e.g., Asians, North Americans, and Europeans),

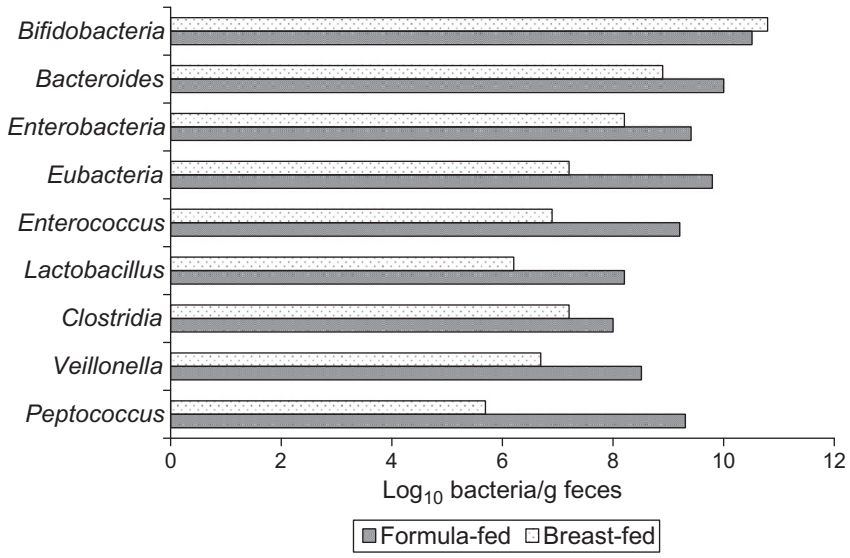

FIGURE 37.10 Comparison of bacterial populations in breast-fed versus formula-fed infants. *Data adapted from Y. Benno, K. Sawada, T. Mitsuoka, T., The intestinal microbiota of infants: composition of fecal flora in breast-fed and bottle-fed infants, Microbiol. Immunol. 28 (1984) 975–986.*

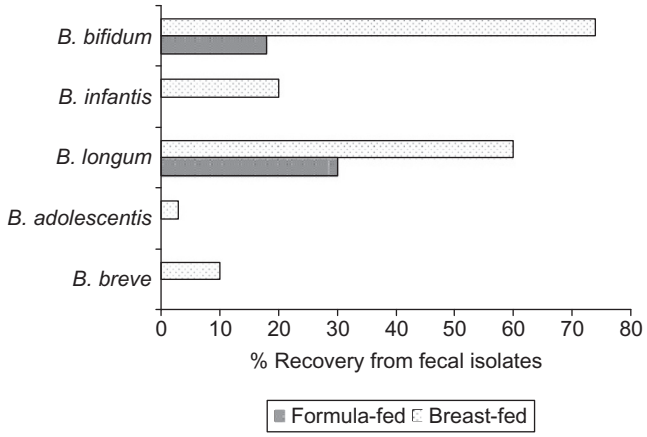

FIGURE 37.11 Differences in the *Bifidobacterium* spp. isolated from fecal samples of breast-fed versus formula-fed infants. *Data adapted from H. Beerens, C. Romond, C. Neut, Influence of breast-feeding on the bifid flora of the newborn intestine, Am. J. Clin. Nutr. 33 (1980) 2434–2439.*

implicating regional dietary habits [37]. Hence, from these observational studies, it appears that dietary patterns influence intestinal microbiota communities.

Intervention studies provide more direct evidence of the ability of diet to modify intestinal microbiota. We conducted unpublished experiments in which diet was strictly controlled and modified from basal, free-living diet. Asian adolescents participated in two 3-week sessions of supervised clinical camp. The "camp" diet comprised food items such as peanut butter-and-jelly sandwiches, pasta, and pizza, but without the addition of probiotics, prebiotics, and other high-fiber food. Meals were given such that all subjects consumed the same types of food at any given meal time (i.e., controlled diet). Changes in intestinal microbiota profile were assessed based on fecal samples using the DGGE method. It was observed that the bacterial community shifts to a different profile within 2–4 days of consuming the new diet and the profile is maintained as long as the diet remains largely unchanged. The intestinal microbiota profiles of these subjects changed from "basal" profile to "camp" profile in the first session of camp, reverted to "basal" profile during the wash-out period, and then slipped back to "camp" profile in the second session of camp [91]. However, intersubject variability remained high, indicating that regardless of dietary changes, each individual maintains his or her unique intestinal microbiota profile [91,92]. Intestinal microbiota profile can also be altered when one type of food is consumed at a higher than usual intake [93,94]. The number of *Bacteroides* spp. and *Staphylococcus* spp. was elevated during periods of high-meat consumption, whereas the number of these bacteria decreased when the diet was devoid of meat. In addition, the number of *Eubacterium* spp., *Bifidobacterium* spp., and *Lactobacillus* spp. was much lower following a high-meat diet compared to a meat-free diet [93]. A high intake of cruciferous vegetables has also been shown to cause a major shift in the intestinal microbiota composition compared to the intestinal microbiota profile of subjects maintained on a fruit- and vegetable-free diet [94].

B Altering the Intestinal Microbiota Using Food Supplements

1 Probiotics

Probiotics contain viable microorganisms that are proposed to improve the health of the host primarily by altering its intestinal microbial communities. Various probiotic bacterial strains are available, but the genera *Lactobacillus* spp.,

Bifidobacterium spp., and *Streptococcus* spp. are the most commonly used. Yogurt, kefir, and capsules containing freeze-dried probiotic bacteria are examples of commercially available probiotics.

The beneficial effects of consuming probiotics, which include improvement in lactose tolerance and a reduction in the risk and severity of diarrheal symptoms (i.e., as a side effect to antibiotics, traveler's diarrhea, or induced by rotavirus as in the case of gastroenteritis), have been observed in clinical studies. Inflammatory bowel disease (IBD), which is characterized by chronic or recurring intestinal inflammation, has been postulated to be caused by abnormal host immune responses to certain members of the intestinal microbiota and/or from a defective mucosal barrier [95]. Some clinical studies have indicated that probiotics can treat IBD (e.g., ulcerative colitis, Crohn's disease, and pouchitis; Table 37.1). Probiotics may also be a treatment for intestinal infection caused by pathogens (e.g., *Clostridium difficile* and *H. pylori*) (Table 37.1). Furthermore, it is hypothesized that regular consumption of probiotics may reduce the levels of secondary bile acids and other mutagens that are involved in colon carcinogenesis [96]. Other studies outlining the efficacy of probiotics in various clinical applications are summarized in Table 37.1.

The efficacy of probiotics remains unproven. Mixed results raise concerns regarding the suitability of the strains, effective dosage, toxicity levels, and the viability of the bacteria in the product. Furthermore, mechanisms by which these bacteria colonize the intestinal tract have not been described [97–99]. Colonization success of the ingested probiotic bacteria is questionable because probiotic bacteria typically are not able to permanently colonize the GI tract. Hence, colonization and benefits are usually transient in nature [100,101].

2 Prebiotics

Prebiotics are defined as nondigestible and fermentable food components that selectively stimulate the growth and/or activity of certain colonic bacteria that bring beneficial health effects to the host. Unlike probiotics, where one attempts to introduce beneficial bacteria into the GI tract, prebiotics attempt to stimulate the growth of endogenous beneficial bacteria by providing specific fermentable substrate(s). Examples of prebiotics are inulin, FOS, GOS, and lactulose.

It has been proposed that a food component has to satisfy the following criteria to be recognized as a prebiotic: (1) resistant to the acidic condition of the stomach, (2) unable to be broken down in the small intestine, (3) transferable to the colon as a fermentable substrate, and (4) able to stimulate the growth of a selective group of beneficial colonic bacteria (e.g., *Bifidobacterium* spp. and *Lactobacillus* spp.) [102]. Clinical studies investigating the effects of prebiotics such as inulin, FOS, GOS, and lactulose are listed in Table 37.2. Although most of these studies resulted in the enhancement of *Bifidobacterium* spp., few of them were able to correlate bacterial data with health-related biomarkers. It is speculated that increases in the colonic levels of *Bifidobacterium* spp. modulate immune function, inhibit pathogen colonization, and augment the production of SCFA (Fig. 37.12).

VII MICROBIOME-ASSOCIATED BIOMARKERS

Given that intestinal microbiota are known to be involved in metabolism and utilization of nutrients, it is not surprising that biomarkers for metabolic syndrome and obesity have been found in the microbiome [103]. A high-fat diet is more likely to result in abundant propionate and acetate producing bacterial species such as *Phascolarctobacterium*, *Proteus mirabilis*, and Veillonellaceae, whereas normal-fat diets are associated with higher prevalence of *Lactobacillus intestinalis*. Furthermore, body weight and fat mass have been found to be negatively correlated with an abundance of *L. intestinalis*, which provides evidence that the composition of the microbiome can help predict obesity risk. Additionally, low bacterial diversity in the gut is associated with increased overall adiposity, triglycerides and free fatty acids, insulin resistance, inflammatory responses, and weight gain over time [104]. These characteristics can then be used to predict the risk of prediabetes, type 2 diabetes, and ischemic cardiovascular disorders.

The gut microbiome also provides biomarkers for asthma, atopic dermatitis, and autoimmune diseases. High fecal calprotectin, a biomarker for intestinal inflammation, was found to be associated with an increased risk of atopic dermatitis and asthma by the age of 6 [105]. It has been proposed that impaired monocyte IL-10 activation due to the lack of *E. coli* colonization is linked to this finding.

VIII CHALLENGES IN THE FIELD

The study of the human intestinal microbiota is a relatively new field. Interest in this field has been stimulated by the active marketing of probiotics and prebiotics for intestinal health. In addition, concerns about the effects of antibiotics (i.e., due to a medical treatment or residues in animal products) have focused research on the GI microbiota.

Given the unknown role that the GI tract plays in maintaining overall health, efforts to advance our knowledge of the complex interaction between host and microbiota are needed. As a first goal, the diversity of the intestinal microbiota needs to be characterized. With better basic knowledge, we can move toward elucidating microbe–microbe and host–microbe interactions. Investigations of the effects of age, gender, host genotype, various components of diet,

TABLE 37.1 Clinical Studies Investigating Various Applications of Probiotics

Intestinal Condition	Probiotic	Reference
Lactose intolerance	*Lactobacillus acidophilus*	[106]
	Lactobacillus acidophilus or *L. casei*	[107]
	Lactobacillus acidophilus	[108]
	Bifidobacterium longum	[109]
	Lactobacillus bulgaricus or *S. thermophilus*	[110]
	Lactobacillus bulgaricus	[111]
	Lactobacillus bulgaricus	[112]
	Lactococcus lactis	[113]
	Lactobacillus casei Shirota	[114]
	Bifidobacterium breve Yakult	[114]
Diarrhea associated with the use of antibiotics	*Saccharomyces boulardii*	[115]
	Lactobacillus acidophilus or *L. bulgaricus*	[116]
	Streptococcus faecium	[117]
	Lactobacillus acidophilus or *L. bulgaricus*	[118]
	Bifidobacterium longum	[119]
	Saccharomyces boulardii	[120]
	Enterococcus faecium SF68	[121]
	Lactobacillus GG	[122]
	Bifidobacterium longum or *L. acidophilus*	[123]
	Saccharomyces boulardii	[124]
	Lactobacillus acidophilus	[125]
	Lactobacillus GG	[126]
	Lactobacillus GG	[127]
	Lactobacillus GG	[128]
	Saccharomyces boulardii	[129]
Rotavirus-induced diarrhea and/or pediatric diarrhea, including gastroenteritis	*Enterococcus faecium* SF68	[130]
	Lactobacillus acidophilus	[131]
	Saccharomyces boulardii	[132]
	Lactobacillus GG	[133]
	Lactobacillus GG	[134]
	Bifidobacterium bifidum or *S. thermophilus*	[135]
	Lactobacillus spp.	[136]
	Lactobacillus GG	[137]
	Lactobacillus GG	[138]
	Lactobacillus GG	[139]
	Enterococcus faecium SF68	[140]
	Lactobacillus GG	[141]
	Lactobacillus GG	[142]
	Lactobacillus reuteri, *Lactobacillus* GG	[143,144]
	Lactobacillus GG	[145]
	Lactobacillus casei DN-114001	[146]
	Bifidobacterium sp. Bb12 or *S. thermophilus*	[147]
	Lactobacillus GG	[148]

(*Continued*)

TABLE 37.1 (Continued)

Intestinal Condition	Probiotic	Reference
	Lactobacillus casei	[149]
	Lactobacillus GG	[150]
	Lactobacillus GG	[151]
	Lactobacillus sporogenes	[152]
	Lactobacillus rhamnosus 19070–2 or *L. reuteri* DSM 12246	[153,154]
	Lactobacillus casei	[155]
	Saccharomyces boulardii	[156]
Traveler's diarrhea	*Lactobacillus acidophilus* or *L. bulgaricus*	[157]
	A mixture of Lactobacilli, Bifidobacteria, and Streptococci	[158]
	Lactobacillus GG	[159]
	Saccharomyces boulardii	[160]
	Lactobacillus GG	[161]
Diarrhea induced by tube feeding	*Saccharomyces boulardii*	[162]
	Saccharomyces boulardii	[163]
	Saccharomyces boulardii	[164]
	Lactobacillus sp.	[165]
Diarrhea in immunocompromised individuals	*Lactobacillus acidophilus*	[166]
	Bifidobacterium sp.	[167]
	Saccharomyces boulardii	[168]
	Lactobacillus reuteri	[169]
	Lactobacillus rhamnosus[a]	[170]
	VSL#3[b]	[171]
Small bowel bacterial overgrowth	*Lactobacillus acidophilus*	[172]
	Lactobacillus acidophilus	[173]
	Lactobacillus plantarum 299 V; *Lactobacillus* GG	[174]
	Lactobacillus casei or *L. acidophilus* Cerela	[175]
Allergic dermatitis	*Lactobacillus* GG	[176]
	Bifidobacterium lactis Bb-12 or *Lactobacillus* GG	[177]
	Lactobacillus GG	[178]
	Lactobacillus rhamnosus GG	[179]
Necrotizing enterocolitis, IBS	*Lactobacillus acidophilus* or *B. infantis*	[180]
	Saccharomyces boulardii	[181]
	Lactobacillus acidophilus	[182]
	Enterococcus faecium M74	[183]
	Lactobacillus acidophilus[c]	[184]
	Enterococcus faecium PRSS	[185]
	Lactobacillus plantarum	[186]
	Lactobacillus helveticus or *L. acidophilus*	[187]
	Escherichia coli[d]	
	Lactobacillus plantarum	[188]
	Propionibacterium freudenreichii	[189]
	Lactobacillus plantarum	[190]

(*Continued*)

TABLE 37.1 (Continued)

Intestinal Condition	Probiotic	Reference
	VSL#3[b]	[191]
	VSL#3[b] *Enterococcus faecium* SF68[e]	[192]
	Lactobacillus plantarum 299 V	[193]
	Bifidobacterium animalis DN-173010	[194]
	Lactobacillus plantarum 299 V	[195]
	VSL#3[b]	[196]
	Bifidobacterium infantis 35624	[197]
	Lactobacillus reuteri DSM 17938	[198]
IBD (e.g., ulcerative colitis, pouchitis, and Crohn's disease)	*Saccharomyces boulardii*	[199]
	Lactobacillus GG	[200]
	Escherichia coli	[201]
	Escherichia coli	[202]
	Escherichia coli	[203]
	VSL#3[b]	[204]
	Saccharomyces boulardii 5-aminosalicylic acid	[205]
	Lactobacillus GG	[206]
	Saccharomyces boulardii	[207]
	VSL#3[b]	[208]
	Lactobacillus GG	[209]
	Lactobacillus GG	[210]
	Escherichia coli Nissle 1917	[211]
	Lactobacillus GG	[212]
	Lactobacillus GG	[213]
Intestinal infection caused by pathogens: *C. difficile*	*Lactobacillus* GG	[214]
	Saccharomyces boulardii	[215]
	Saccharomyces boulardii	[216]
	Saccharomyces boulardii	[217]
	Saccharomyces boulardii	[218]
	Lactobacillus GG	[219]
	Lactobacillus GG	[220]
	Lactobacillus plantarum 299 V	[221]
Intestinal infection caused by pathogens: *H. pylori*	*Lactobacillus acidophilus* (Johnsonii) La1	[222]
	Lactobacillus acidophilus	[223]
	Lactobacillus gasseri 2716 (LG21)	[224]
	Citrobacter rodentium	[225]

[a] *Antibiophilus.*
[b] *VSL#3 contains four strains of lactobacilli (*L. casei, L. plantarum, L. acidophilus*, and* L. delbruekii *subsp.* bulgaricus*), three strains of bifidobacteria (*B. longum, B. breve*, and* B. infantis*), and one strain of* Streptococcus salivarius *subsp.* thermophilus.
[c] *Lacteol Forte.*
[d] *Hylac N and Hylac N Forte.*
[e] *Bioflorin.*

TABLE 37.2 Clinical Studies Investigating Alterations in Intestinal Microbiota as a Response to Prebiotics

Prebiotic	Dose and Duration	Microbiota Modulation	Reference
Inulin	8 g/day, 14 days	Increase in bifidobacteria, slight increase in clostridia	[225]
Inulin	Up to 34 g/day, 64 days	Increase in bifidobacteria	[226]
Inulin	15 g/day, 15 days	Increase in bifidobacteria	[227]
Inulin	20 g/day, 19 days	Increase in bifidobacteria, decrease in enterococci and enterobacteria	[228]
FOS	15 g/day, 15 days	Increase in bifidobacteria, decrease in *Bacteroides*, clostridia, and fusobacteria	[229]
FOS and PHGG	6.6 g/day FOS, 3.4 g/day PHGG, 21 days	Increase in bifidobacteria	[230]
FOS	0–20 g/day, 7 days	Increase in bifidobacteria	[231]
FOS	4 g/day, 42 days	Increase in bifidobacteria	[232]
FOS + GOS	0.04 g/L, 0.08 g/L, 28 days	Increase in bifidobacteria and lactobacilli	[233]
FOS + GOS	10 g/L, 28 days	Increase in bifidobacteria	[234]
Lactulose	10 g/day, 26 days	Increase in bifidobacteria	[235]
Lactulose	3 g/day, 14 days	Increase in bifidobacteria, decrease in lactobacilli	[236]
Lactulose	2 × 10 g/day, 4 weeks	Increase in bifidobacteria and lactobacilli	[237]
Lactulose	5 g/L and 10 g/L, 3 weeks	Increase in bifidobacteria, decrease in coliforms	[238]
GOS	0–10 g/day, 8 weeks	Increase in bifidobacteria and lactobacilli	[239]
GOS	2.5 g/day, 3 weeks	Increase in bifidobacteria, decrease in *Bacteroides* and clostridia	[240]

FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; PHGG, partially hydrolyzed guar gum.
Source: Reprinted with permission from K.M. Tuohy, G.C.M. Rouzaud, W.M. Bruck, G.R. Gibson, Modulation of the human gut microbiota towards improved health using prebiotics: assessment of efficacy, Curr. Pharm. Des. 11 (2005) 75–90.

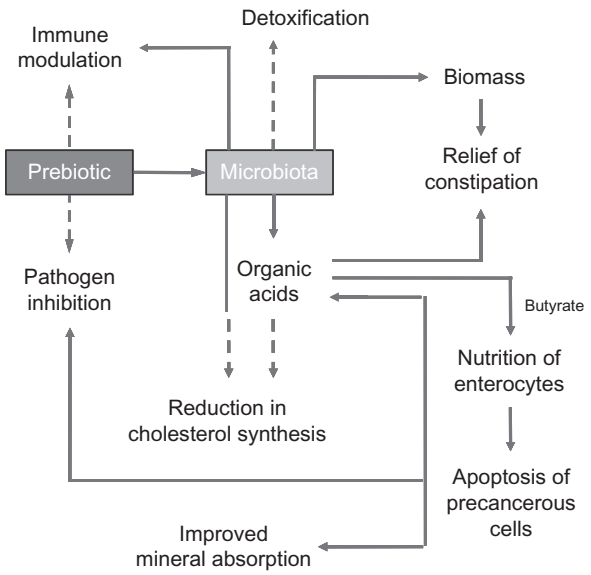

FIGURE 37.12 Possible mechanisms of prebiotic action. Solid lines indicate well-established modes of action, and dotted lines indicate speculative mechanisms. *Modified from A.C. Ouwehand, M. Derrien, W. de Vos, K. Tiihonen, M. Rautonen, Prebiotics and other microbial substrates for gut functionality, Curr. Opin. Biotechnol. 16 (2005) 212–217 [246].*

and the environment on intestinal microbial community are additional goals. Reliable methods to detect alterations in the composition of the intestinal microbiota are required to meet these objectives. Molecular methods hold much promise in this regard [72,73].

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Chapter 38

Gut Microbial Metabolism in Health and Disease

Wei Jia[1,2] and Cynthia Rajani[1]

[1]University of Hawaii Cancer Center, Honolulu, HI, United States, [2]Shanghai Jiao Tong University School of Medicine, Shanghai, China

I INTRODUCTION TO THE GUT MICROBIOTA

In the early 1980s, researchers at the University of Notre Dame, Indiana, reported that germ-free (GF) mice could survive without microbe colonization; however, they had higher nutritional demands in terms of quantity of food and composition [1]. GF mice required 30% more energy in their diet as well as supplementation with vitamins K and B to maintain their body weight. Furthermore, they did not develop normal anatomy or physiology and were more susceptible to infections. Combined, these effects provided clear evidence that gut bacterial communities were important components of the host defense barrier [2]. GF mice were found to have smaller intestines of decreased surface area, thinner lamina propria (LP), larger cecums, fewer plasma cells and intraepithelial lymphocytes, lower IgA levels, as well as underdeveloped secondary lymphoid structures such as smaller Peyer's patches and mesenteric lymph nodes than conventional animals [3]. Thus, while microbial colonization is not essential for life, it is important for normal growth, development, and immunity.

Knowledge that the gut microbiota may be important for human health prompted scientists to seek ways to identify and classify those microbes present in the gastrointestinal (GI) tract. Amongst other factors, the strict anaerobic nature and the symbiotic interactions between species of bacteria and the host have made it challenging to culture beyond a minority of these organisms. In 1977, Woese and Fox made the discovery that the nucleotide sequence of 16S ribosomal RNA (16S rRNA), a component of the 30S small subunit of prokaryotic ribosomes, present in every bacterial and archaeal cell, contained regions that were highly conserved and that these sequences flanked regions that were variable among the different bacterial species which allowed their phylogenetic classification [4]. Only recently have high-throughput sequencing techniques, such as phylogenetic DNA microarray, whole genome, and next-generation sequencing, been developed which allow taxonomic classification of gut bacteria via comparison with 16S rRNA sequence databases such as the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/nucleotide) [5].

The classical understanding of inflammation is that of a normal physiologic response to an infectious threat or tissue injury that leads to tissue repair, resolution, and restoration to original tissue homeostasis. Chronic, noncommunicable inflammatory diseases such as metabolic syndrome (MS), type 2 diabetes (T2D), irritable bowel syndrome (IBS), obesity, and neurodegenerative disorders do not really fit this model as there is no acute threat nor is the inflammation resolved. Instead, there is an adaptation to a new set of conditions, a "shift" in the balance perhaps brought about by a change in diet, overuse of antibiotics, or other environmental insults that alter the nutritional interrelationship between the host and microbiota metabolisms. This adaptation results in altered gut colonization patterns or "dysbiosis," a common, central theme found in these chronic inflammatory conditions [6]. In this chapter the relationship between the gut microbiota and incidence of chronic noncommunicable inflammatory diseases will be reviewed including a discussion on the impact of microbial metabolism and metabolomics approaches used in these investigations.

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A The Gut Microbiota and Diet

Changes in dietary patterns and habits are some of the most important lifestyle decisions that can affect a person's health. These changes are second only to cessation of smoking for disease prevention [7]. It is believed that a healthy population of commensal bacteria is essential for optimal host−microbiota mutualism and overall human health. Thus, an increasingly important question is how can the microbiota be shaped throughout life and from meal-to-meal to prevent, cure, or alter the progression of a disease? Overall, dietary fiber is the main source of energy for bacteria in the colon [8]. As such, a key factor that affects the composition of microbiota is the availability of glycans (derived from fiber) in the lower intestine. Human hosts are capable of fully digesting only starch, lactose, and sucrose glycans each of which contain only one or two different linkages. However, a much more diverse array of glycans can be derived from the diet in the form of both plants and animal sources. Microbial fermentation of these otherwise indigestible glycans converts them into short-chain fatty acids (SCFA) which are well known to serve as nutrients for the GI epithelial cells. Individual microorganisms are able to preferentially metabolize different glycans and thus, selective human consumption could potentially influence which microbiota populations will flourish. Whereas dietary glycans may vary, endogenous *N*-linked glycans produced by shed GI epithelial cells and *O*-linked glycans attached to mucus cells provide a more stable source. However, the ability of certain microbes to interact closely with the mucosal layer and utilize endogenous glycans may negatively affect mucosal barrier function and colonic health, especially under conditions of dysbiosis [9].

The human gut microbiota is initially seeded from the microbes presented at birth and is believed to become established within the first few days. Breast-fed infants are exposed to a unique mixture of complex human milk oligosaccharides (HMOs) not present in the milk of other mammals. These HMOs are not digested by human enzymes and, it has been suggested, that they evolved to serve as natural prebiotics and guide development of infant microbiota by serving as selective substrates for specific bacterial species and communities (e.g., *Bifidobacterium longum* biovar. *infantis*) [10]. The fact that HMOs are indigestible by the infant through normal digestion means they will be the most abundant macromolecule reaching the lower intestine and those bacteria that can selectively utilize these substrates to aid in establishing specific community profiles will thrive. HMOs are complex, containing a diverse array of sugars. *Lactobacilli* sp., also preeminent in the infant gut, prefers monosaccharide components of the HMOs suggesting that selection of individual components of HMO may contribute to broader microbial community structure [9]. Formula-fed (cow's milk) infants were found to have lower amounts of *Lactobacilli* and *Bifidobacteria* but showed increased abundances of *Clostridium*, *Bacteroides*, and members of the Enterobactericeae. Cow's milk lacks the diversity and amounts of oligosaccharides when compared to human milk and does not contain the unique HMOs [9,11].

At approximately 6 months, the composition of the human diet begins to incorporate complex foods, such as cereals, fruits, and vegetables, that diversify the digestible and indigestible carbohydrate portion of the diet. This in turn induces a shift in the microbiota to gram-negative Bacteriodetes and other species of Firmicutes become more abundant. These two phyla numerically dominate the adult microbiota. When a fully omnivorous diet is established, there is microbiota stabilization with fewer temporal changes, although the ratio of Bacteriodetes/Firmicutes can change with diet. A third phylum, Actinobacteria, is also frequently found in adult microbiota [9].

Direct evidence for the effect of diet on gut microbiota was reported in a study that involved two populations of children. One was a population from Burkina Faso (BF), who had a diet high in fiber content similar to that of early human agricultural settlement and was compared to European (EU) children on a typical western, high fat/low fiber diet. The 16S rRNA sequencing and biochemical analysis revealed that BF children had enriched amounts of Bacteroidetes and depletion of Firmicutes with an abundance of *Prevotella* and *Xylanibacter*. The last phylum contains many genes for cellulose and xylan hydrolysis and was completely lacking in EU children. Significantly higher concentrations of SCFAs were found in BF children along with significantly reduced populations of *Shigella* and *Escherichia* which would be protective against inflammation (Fig. 38.1) and irritable bowel disease (IBD) [12].

Dietary supplementation with particular carbohydrates may be another means of eliciting changes in species abundance. Prebiotics are defined as a selectively fermented substance, typically carbohydrates, that allows specific microbiota changes that confer benefits upon host health. Resistant starch (RS) is an example of a potential prebiotic dietary component. RS is dietary starch that is inaccessible to human digestive enzymes and therefore traverses to the colon and is made available to the microbiota. RS can be divided into four types: (1) RS1, starch found in whole grains or partially milled grains and legumes; (2) RS2, granular starch that is tightly packed and dehydrated; (3) RS3, cooked and reassociated starch with limited accessibility to host enzymes, and (4) RS4, chemically or enzymatically modified starch. Within the RS3 type, different X-ray diffraction patterns based on amylose content have also been found which favor particular bacteria. For example, typical cereal starches exhibit an A-type pattern (*Atopobium* spp.) while tuber and other starches with high amylose content favor a B-type pattern

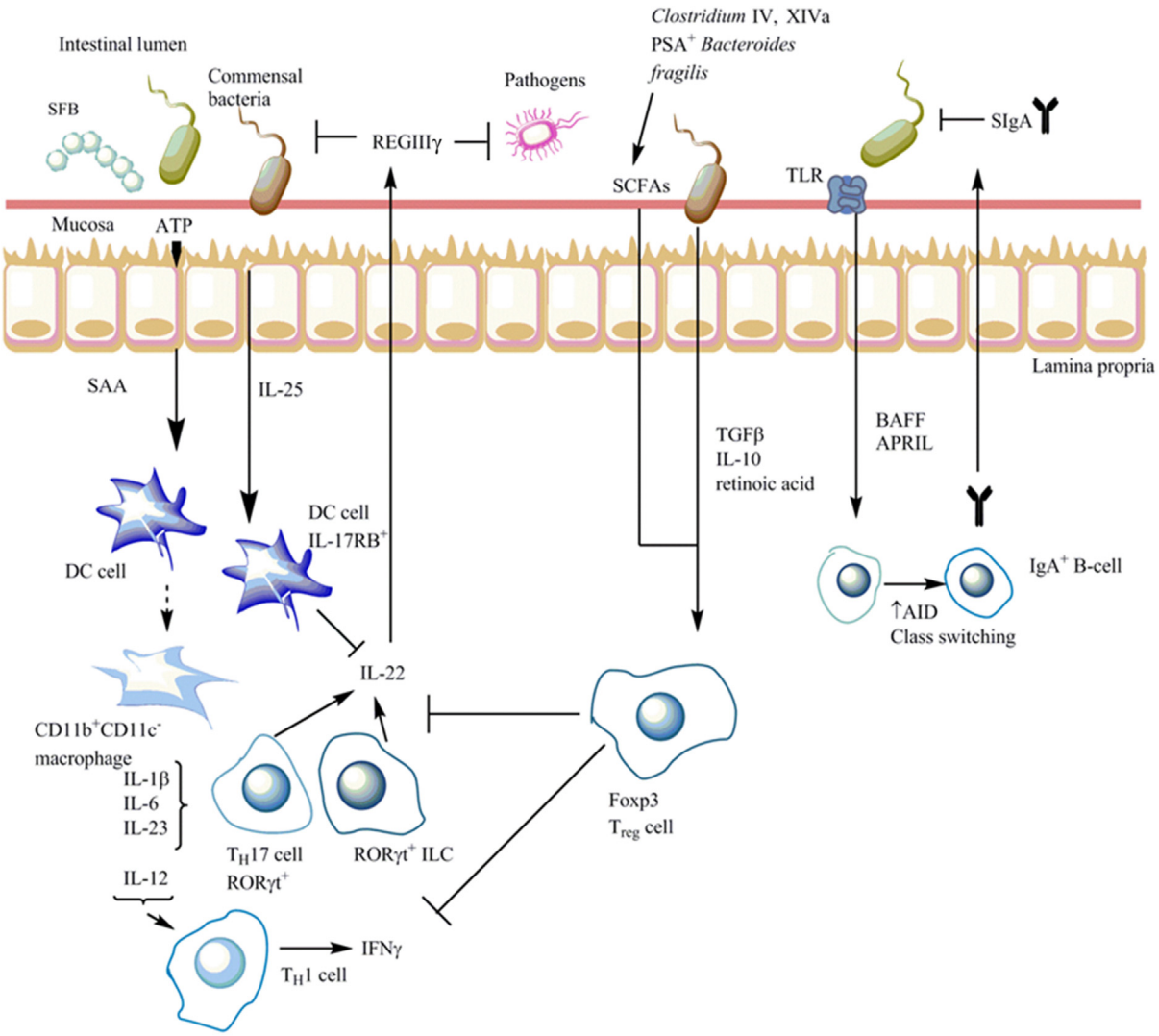

FIGURE 38.1 Gut microbiota shape the immune system to promote microbiota−host mutualism. Abbreviations: IL, interleukin; T_H, T-helper cell; T_{reg}, T-regulatory cell; PSA, polysaccharide A (see text also).

(*Bifidobacterium* spp.). B-type pattern starches favored higher butyrate production indicative of differences in the bacterial species that were enhanced with this supplementation [8]. Based on human feeding studies, RS supplementation was also reported to increase the amount of butyrate, a common metabolite of Firmicutes, that is, not only a nutrient for the mucosal epithelium but is also thought to be antiinflammatory via its ability to increase differentiation of T_{reg} cells (Fig. 38.1) [13].

Hemicellulose is another major category of dietary fiber that includes arabinoxylans, xyloglucans, glucomannans, galactomannans, and β-glucans. Arabinoxylooligosaccharides (ARS), the hydrolyzate of arabinoxylan and β-glucan have been shown to stimulate the growth of *Bifidobacteria* spp. in the human colon and thus there is interest in using them as prebiotics. In a simulator of intestinal microbial ecosystem (SHIME), the degree of polymerization (DP) of ARS was found to influence which species of bacteria fermented it and where fermentation occurred in the colon [14]. Interestingly, in a protein-rich environment, decreases in the proteolytic markers, phenol and *p*-cresol, were discovered and continued to decrease after the 3-week treatment with arabinoxylin-oligosaccharides (AXOS) was discontinued indicating a longer term effect on microbiota. Proteolysis can lead to higher levels of tryptophan (TRP) and increased inflammation via its metabolism along the TRP → KYN pathway (Fig. 38.2). Increased amounts (25−48%) of two beneficial SCFAs, butyrate and propionate, were also observed in all colonic vessels [14]. Higher DPs (≥60%) in humanized rats provided some different results highlighting the importance of molecular size for the efficacy of AXOS as a prebiotic [15]. The molecular size of β-glucan has also been shown to play

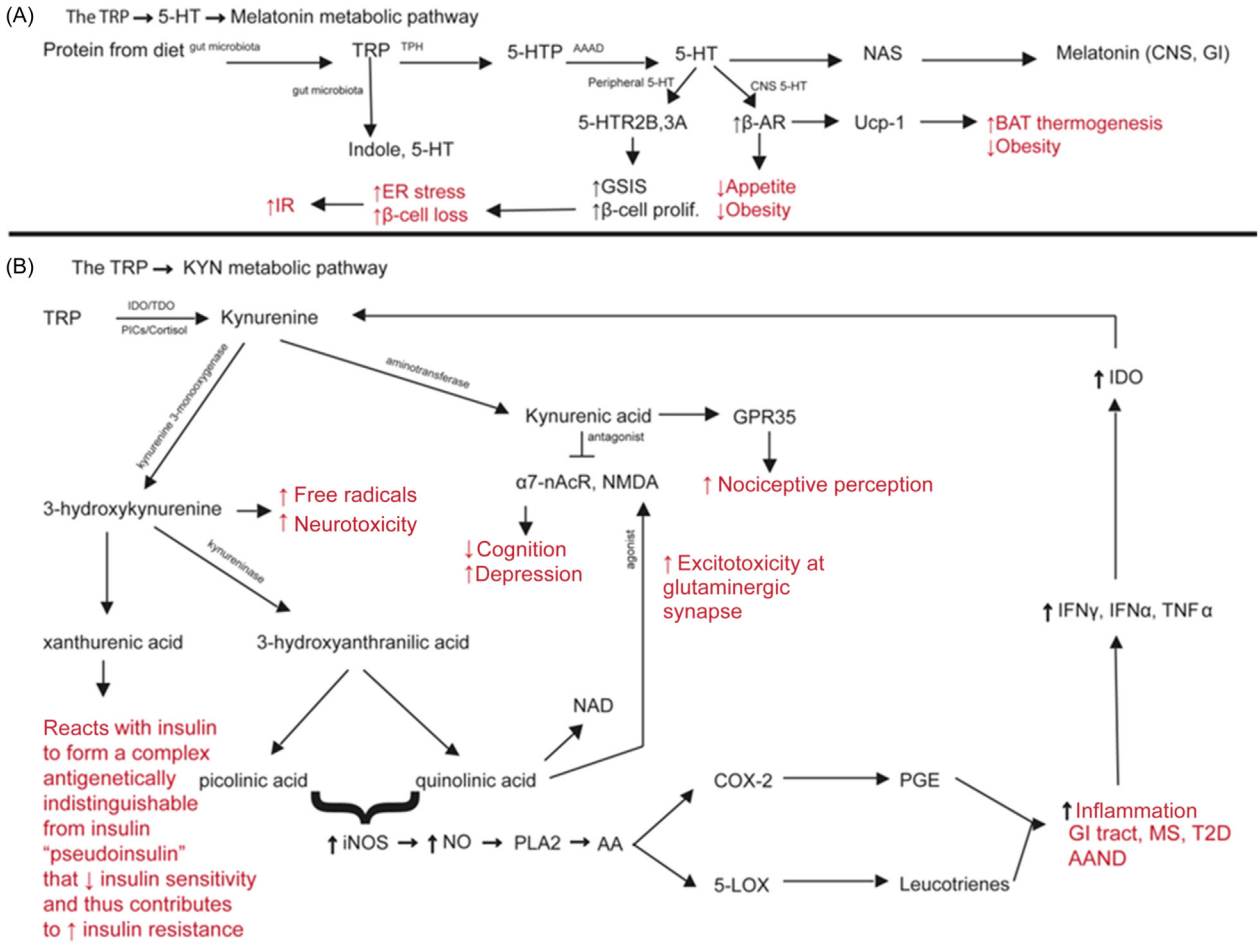

FIGURE 38.2 (A) The TRP → 5-HT → Melatonin metabolic pathway. (B) The TRP → KYN metabolic pathway. Abbreviations: NAS, *N*-acetylserotonin; 5-HTR2B,3A, 5-HT receptor 2B,3A; β-AR, β-adrenergic receptor; Ucp-1, uncoupling protein-1; BAT, brown adipose tissue; GPR35, G-protein coupled receptor-35; NAD, nicotinamide adenine dinucleotide; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PLA2, phospholipase A2; AA, arachidonic acid; COX-2, cyclooxygenase-2; PGE, prostaglandin; 5-LOX, arachidonate 5-lipoxygenase; AAND, age-associated neuroendocrine disorders; IFN, interferon; TNFα, tumor necrosis factor-α.

a role in favoring specific bacterial groups (Table 38.1). Fructans can also be used as prebiotics and comparison studies have been performed for inulin (DP 3−60) and one of its enzymatically hydrolyzed products, oligofructose (DP 2−20) using the SHIME. Inulin took longer to establish a prebiotic effect but its effect was more pronounced with respect to fermentation activity and microbial community composition changes than was seen with the shorter chain oligofructose (Table 38.1) [16].

It is important to consider that dietary carbohydrates and other nutrients can fluctuate from meal-to-meal. Recently, human subjects fed either high-fat/low-fiber or low-fat/high-fiber diets in a controlled experiment showed detectable shifts in microbiota after just 24 hours suggesting that some proportion of human microbiota are constantly fluctuating in response to feeding [17]. Another important factor with respect to meals and potentially microbiota is circadian rhythm. It has been shown that not only amount and content of food but time of ingestion is also critical for health. A well-known medieval quote is as follows, "Eat like a king in the morning, a prince at noon, and a peasant at dinner." Because a large fraction of the microbiota is located in the intestine which also has a powerful circadian clock that participates in daily cycles of food digestion, it is not surprising that studies have shown that the intestinal epithelial cells (IECs) circadian clock can be disrupted by depletion of microbiota. Therefore, changes in microbiota induced by different dietary and life patterns likely differentially regulate the gut microbial communities and circadian rhythm [18].

In summary, the lower intestine harbors a large portion of the overall gut microbiota. Undigestible carbohydrates (fiber) have been shown to be influential in both shaping and maintaining a healthy gut microbiota. This has led to the formulation of dietary supplements and foods with prebiotics to aid individuals in the establishment and

TABLE 38.1 The Effect of Molecular Size of Hydroxylates of AXOS, β-Glucan, Inulin, and Oligofructose on Microbiota

AXOS			
DP or MW	**Colon Location**	**Bacterial Effect**	**Other**
29% [14]	Ascending colon	↑*Bifidobacteria* spp.	↑butyrate
	Ascending, transverse	↑*Lactobacilli* spp.	↑propionate
	Descending	↑*Clostridium coccoides*	↓phenol
		↑*Eubacterium rectale*	↓p-cresol
≥60% [15]	Cecum	↓Clostridia clusters I/XI/XV	↑SCFAs
		↓*Verrucomicrobia*	
		↑Actinobacteria	
		↑*Bifidobacterium longum*	
β-Glucan:			
137–172 kDa		↑*Bacteriodes*	
230–243 kDa		↑Prevotella	
		No increases seen	
Inulin [16]:			
3–60%		↑*Lactobacilli* spp.	47% ↑SCFAs
	Ascending	↑*Bifidobacteria*	18–33% ↓NH_3
Oligofructose [16]:			
2–20%	Ascending, descending	↑*Bifidobacteria*	28% ↑SCFAs
	Ascending, descending	↓*Lactobacilli* spp.	10–13% ↑NH_3
			↑BCFAs (from protein)

NH_3, ammonia; BCFAs, branched-chain fatty acids; MW, molecular weight.

maintenance of "healthy" microbiota. An imbalance in this system, gut dysbiosis, is a central theme in the non-communicable inflammatory diseases, obesity, IBS, mental health, and age-related neurodegeneration disorder (ARNDD) that will be discussed further.

B The Gut Microbiota and the Mucosal Barrier Immune System

Mucosal surfaces in the intestine represent the largest area through which microorganisms and the environment interact with the immune system. The recognition that the gut microbiota impact not only metabolism and immunity in the intestine but also systemically including the brain has given rise to the concept of a gut-liver and gut-brain axis in humans [19]. Microbiota are involved in microbe–host epithelium interactions which have the effect of shaping intestinal immune systems. Fig. 38.1 summarizes how the gut microbiota control key populations of dendritic cells (DCs) that give rise to three major classes of T-cells, T_H1, T_H17, and T_{reg}, as well as an important IgA secreting type of B-cell. Microbiota-induced intestinal immunity functions to prevent overgrowth of bacteria and prevents colonization by pathogens thereby maintaining the integrity of the mucosal barrier.

A central strategy employed by the host is to minimize contact of both pathogens and commensal microbes with the epithelium. To control bacterial overgrowth, segmented filamentous bacteria (SFP) adhere to the host epithelium and upregulate serum amyloid protein A (SAA). SAA is then actively transported using ATP and triggers the differentiation of particular DCs into $CD11b^+/CD11c^-$ macrophages which produce IL-1β, IL-6, and IL-23 that effect T-cell differentiation into T_H17 cells. Alternatively, these same macrophages can produce IL-12

which causes T-cell differentiation to T_H1 cells. T_H17 cells produce IL-22 that interacts with epithelial enterocytes to produce regenerating islet-derived protein 3γ (REGIIIγ), an antibacterial protein, that blocks microbial proliferation via a proinflammatory immune response. IL-22 can also be produced by innate lymphoid cells (ILCs) that express retinoic acid receptor-related orphan receptor-γt (RORγt+) to act on epithelial REGIIIγ production. T_H1 cells produce antiviral interferon gamma (IFNγ). Microbes such as *Clostridium cluster IV*, *VIVa* strains and PSA^+ *B. fragilis* can adhere to the epithelium and activate the transforming growth factor β (TGFβ) pathway or, alternatively, activate DCs to produce retinoic acid and IL-10 which together promote T-cell differentiation to Forkhead box-P3 ($Foxp3^+$) T_{reg} cells. T_{reg} cells negatively regulate the effects of T_H17 cells by causing antiinflammatory immunosuppression thus preserving host−microbe homeostasis [20−22]. Carbohydrate metabolites including the SCFA butyrate produced by commensal microbiota such as the *Clostridium* IV cluster can selectively expand the population of $Foxp3^+$ T_{reg} cells [23].

Prevention of pathogen colonization relies not only on protection via antibacterial peptides such as REGIIIγ, but also via regulation of B-cell differentiation [24]. B-cells that differentiate to secrete IgA are important for mucosal barrier integrity. When IgA is secreted into the lumen (soluble IgA, SIgA) both commensal bacteria and other soluble antigens are coated to prevent their binding and penetration of the epithelium [25]. In the intestine, binding of bacteria to toll-like receptors (TLRs) on the surface of the epithelium leads to secretion by the epithelial cells of two important factors, B-cell activating factor (BAFF) and a proliferation-inducing ligand, APRIL, to promote the differentiation of B-cells via their increased expression of activation-induced cytidine deaminase to an IgA^+ B-cell [20,25].

Comparison between GI homeostatic and inflammatory conditions (Table 38.2) shows a definite shift in the ratios of T-cell subtypes. Inflammation results in increased numbers of T_H17 cells and suppression of T_{reg} cells, IL-10, and REGIIIγ. In IBD there is an increase in the T_H17/T_{reg} ratio [20]. The result is increased commensal bacteria loss (gut dysbiosis) [26]. Under homeostatic conditions, intestinal macrophages are hyporesponsive, but respond to overproduction of microbial products by secreting proinflammatory cytokines (PICs) [20,27].

TABLE 38.2 Comparison Between Intestinal Homeostasis and Inflammation [20]

Homeostasis (Commensal)	Inflammation (Dysbiosis)
Proper GALT development	↑↑↑T_H17 and T_H1 cells
↑T_H17 and T_H1 cells	↓T_{reg} cells
↑Barrier function	↓IL-10
↑REGIIIγ	↓REGIIIγ
↑T_{reg} cells and IL-10	↑Pathobiont growth
↓Growth of pathobionts	↑PICs IL-1β, IL-6, TNFα

C The Gut Microbiota and TRP Metabolism

Serotonin (5-HT) is a key neurotransmitter that allows bidirectional communication between the central nervous system (CNS) and the GI tract. There is strong evidence that gut microbial influence on TRP metabolism is an important regulator of the "gut-brain" axis [28]. Table 38.3 lists the important effects of serotonergic metabolism.

TRP, an essential amino acid obtained from the diet, is first proteolyzed from ingested protein by gut microbiota (Fig. 38.2A) and then converted by GI enterochromaffin (EC) cells to 5-HT:

$$\text{TRP} \xrightarrow{\text{TRP hydroxylase}} \text{5-hydroxy-tryptophan (5-HTP)} \xrightarrow[\text{(AAAD)}]{\text{aromatic amino acid decarboxylase}} \text{5-HT} \rightarrow \text{Melatonin}$$

Certain bacterial species including *Lactobacillus plantarum*, *Streptococcus thermophilis*, *E. coli K-12*, and *Klebsiella pneumoniae* have TRP synthase enzymes and can directly produce 5-HT from TRP [29]. However, the main fate of TRP is not 5-HT but rather 95% ends up being shunted down the kynurenine (KYN)

TABLE 38.3 Important Physiologic Effects of Serotonergic Metabolism [28]

Behavioral Effects	CNS Effects	GI Effects
Visceral pain	Motor control	Gastric secretion
Emotion	Circadian rhythm	GI motility
Appetite	Cerebellar regulation	GI secretions
Addiction	Body temperature	Colonic tone
Sexuality	CNS vascular tone	Pancreatic secretion
Stress response		Emesis
Cognition		
Sleep		

metabolic pathway (Fig. 38.2B) [28]. The rate-limiting enzyme for KYN formation is indoleamine 2,3-dioxygenase (IDO) which is ubiquitously expressed except in liver, astrocytes, microglia, microvascular endothelial cells, and macrophages. The other enzyme that can convert TRP to KYN is TRP 2,3-dioxygenase (TDO) which is expressed mostly in the liver but is also found in kidney and brain. IDO is activated by PICs and TDO is activated by stress hormones such as cortisol. KYN metabolism leads to four important acids and production of NAD. Xanthurenic acid, which has been implicated in insulin resistance (IR), kynurenic acid, an antagonist for both α7-nicotinic-acetylcholinergic (α7-nAcR) and *N*-methyl-D-aspartate (NMDA) receptors and picolinic/quinolinic acids which are known to produce PICs as well as NAD [30].

The use of GF mice has revealed that the gut microbiota is essential for normal brain development and behavior. Relative to conventional (CON) mice, GF mice had increased plasma levels of TRP and 5-HT and a decreased KYN/TRP ratio (used as an indicator of IDO or TDO enzyme activity) which was normalized following introduction of microbiota [31]. Administration of *L. johnsonii* to rats was found to reduce serum KYN concentrations along with an increase in hydrogen peroxide (H_2O_2) in their ileum lumens. Further testing of H_2O_2 on IDO levels in HT-29 human IECs showed decreased enzyme activity that was attributed to oxidation and inactivation of the enzyme [32]. Given that many *Lactobacillus* sp. and other lactic acid producing bacteria can produce H_2O_2, this is a plausible way in which the microbiota can affect TRP metabolism. Comparisons have also been made between GF, specific pathogen-free (SPF) and CON mice with respect to motor ability and anxiety behaviors. It was found that GF mice had less anxiety and traveled longer distances at both slow and fast speeds than SPF and CON mice. There were no differences between SPF and CON mice for any of the tests. Introduction of microbiota early in GF mice produced CON mice that behaved like SPF mice whereas when adult GF mice were conventionalized, they did not become like SPF indicating that there was a window for achieving certain behavior patterns. The differences found in this study were increased amounts of two key proteins related to synaptic vesicle maturation, synaptophysin, and PSD-95 which were increased in early CON mice but not those conventionalized as adults. Early CON mice also had 2.8-fold increases in plasma 5-HT levels relative to GF mice. This postnatal change induced by microbiota may thus change peripheral 5-HT pools in ways that influence behavior and brain development in an early-life sensitive window [33].

The gut microbiota can also directly utilize TRP thus limiting its availability to the host. Certain bacterial strains such as *B. fragilis* can produce indole from TRP via tryptophanase enzymes [34].

$$\text{TRP} \xrightarrow{\text{tryptophanase}} \text{Indole} + \text{Pyruvate} + \text{Ammonia}$$

Prokaryotes, unlike eukaryotes, can also synthesize TRP utilizing TRP synthase enzymes and thus provide an additional source of TRP to the host [28,35]. Certain strains of *Lactobacilli*, *Streptococci*, *E. coli*, *Morganella* and *K. pneumoniae* can produce 5-HT from TRP [28,29]. It has also been reported that certain gram-positive bacteria are sensitive to increased concentrations of 5-HT. This is evidenced by their negative response to administration of selective serotonin reuptake inhibitors [36]. Therefore, TRP levels available to the host are not only derived directly from the diet but also rely on the balance between microbial TRP utilization and metabolism, microbial 5-HT production, direct microbial synthesis of TRP, and antimicrobial response to exogenous 5-HT. Finally, because the TRP-KYN pathway is driven by PICs (Fig. 38.2) and the microbiota control the mucosal production of PICs such as TNFα, IFNγ, IL-1β, IL-23, IL-25 and IL-6 (Fig. 38.1), they can indirectly control TRP metabolism along the KYN pathway via their role in mucosal immunity.

D Gut Microbiota and Bile Acid Metabolism

As noted previously, gut microbial compositions can be altered by diet, antibiotic treatment, and presence of disease. Bile acids (BAs) appear to be a major regulator of the commensal microbiota. The liver is responsible for the synthesis of hydrophilic, glyco- or tauro-conjugated primary BAs [37]. Commensal bacteria, via bile salt hydrolase enzymes, convert the primary conjugated BAs of hepatic origin into unconjugated BAs, thereby reducing their toxicity allowing for improved bacterial survival and growth in the colon [38,39]. In the colon, select *Clostridium* species further transform the primary unconjugated BAs, chenodeoxycholic acid (CDCA) and cholic acid (CA) into the secondary BAs, lithocholic acid (LCA) and deoxycholic acid (DCA) [38].

BA pool size and composition appear to be important factors in regulating mammalian gut microbial community structure. BAs have direct antimicrobial effects on gut microbiota [40] and may affect microbial compositions through farnesoid-x receptor (FXR)-induced antimicrobial peptides (AMPs) [41] and metabolism of BAs. For example, the microbial metabolite of CA, DCA has antimicrobial activity an order of magnitude greater than that of CA [40]. Rapid and significant changes in the microbiome community structures are observed when rats are fed BAs. Increased BAs in the gut appear to favor gram-positive members of the Firmicutes, such as *Clostridium*

cluster XVIa, and conversely, decreased intestinal levels of BAs lead to a depletion of this taxonomic group in the gut.

While the composition of the mammalian microbiota is a product of nature, it is clear that our environment, particularly diet and lifestyle, shapes the composition of our commensal microbiota. High fat diets (HFDs) increase the levels of BAs excreted to the intestine. Such increase in BA concentration in the large intestine leads to an increase of taxa including BA 7α-dehydroxylating species. On the other hand, an altered gut microbial community structure will profoundly impact BA metabolism that are central to regulation of lipid and glucose metabolism.

Evidence for the effect of commensal bacteria on BA synthesis originated from studies of mice raised in the absence of commensal bacteria where fewer secondary and more conjugated BAs presented in fecal matter [42,43]. Convergent data for human IBD, a condition characterized by diminished microbial diversity and decreased amounts of bacteria of the Firmicutes phylum especially, *Faecalibacterium prausnitzii* showed significantly higher conjugated and lower secondary BA levels relative to healthy controls. Interestingly, increased amounts of 3-OH-sulfated secondary BAs were also detected [44–46]. Sulfation eliminates the ability of secondary BAs to activate the antiinflammatory BA receptor, FXR [43]. The BAs act as receptor agonists for FXR with the following rank order: CDCA > LCA = DCA > CA [39,47]. Therefore, loss of commensal bacteria may result in a diminished ability to produce unconjugated and secondary BAs that are preferred ligands for FXR, as well as increased BA deactivation via sulfation. This can lead to a negative effect on the ability to control intestinal inflammation. On the other hand, retention of commensal bacteria such as Firmicutes species (i.e., *Clostridium* sp.) allows for increased production of butyrate and subsequent promotion of antiinflammatory T_{reg} cell expansion and important nutrition for the enteric mucosal cells [23].

Hepatic FXR activity is normally responsible for maintaining BA levels in the biliary tree and intestine while limiting BA accumulation in the liver. Normal intestinal FXR activity causes increased efflux of BAs back into the portal vein and decreased BA reuptake via apical sodium-dependent bile salt transporters (ASBT) into the enterocyte and thus limits the amount of BAs in the enterocytes [48]. Decreased FXR signaling in the enterocyte can result in a decreased expression of the BA carrier protein, ileal-BA binding protein (I-BAP) which acts to shuttle BAs from the luminal to the serosal side of the intestinal enterocyte. This is combined with the ability to downregulate organic solute transporters-α and -β. The result is reduced flow of BAs back into the portal vein and accumulation of BAs in the intestinal enterocyte. There is also decreased fibroblast growth factor (FGF19) production which causes an increase of BA synthesis in the liver which in turn contributes to cholestasis and hepatic inflammation via nuclear factor kappa B (NF-κB) activation, PIC production, and subsequent inhibition of hepatic FXR. Decreased FXR signaling in the liver causes decreased expression of most of the BA transporters and diminishes BA transport both into and out of the liver resulting in poor enterohepatic circulation. This disruption of BA transport in combination with increased BA synthesis in the liver ultimately leads to cholestasis in the liver and an increased degree of hepatic inflammation [49,50].

E Gut Microbiota and Obesity

The first evidence for the influence of microbiota on body mass came from studies using GF mice which consumed 30% more food to maintain a specific weight. However, upon colonization with microbiota these mice experienced a body weight increase of 60% within 2 weeks. In addition, GF mice fed a high fat, sugar-rich diet were protected from diet-induced obesity and had better insulin sensitivity [51,52]. One important difference between GF and CON mice is the increase in energy harvest that is possible due to the microbiota's ability to ferment complex dietary polysaccharides to SCFAs which not only provide 10% of daily energy supply but also act as signaling molecules that may influence appetite and metabolism. The IEC expressed G-protein coupled receptors, GPR43 and GPR41, play important roles in host–microbiota mutualism (Fig. 38.3A). GPR43 when activated by either of the SCFAs, acetate or propionate, promotes secretion of the hormone, glucagon-like peptide-1 (GLP-1). This hormone is known to increase insulin release and decrease glucagon secretion resulting in a decrease in blood glucose. GLP-1 acts to decrease GI motility potentially resulting in an increase in energy harvest and feelings of satiety [53]. GPR41, which is activated by butyrate or propionate, increases plasma levels of peptide YY (PYY) that also has the effect of decreasing gut motility and increasing energy harvest and feelings of satiety [52]. Activation of either of these two receptors causes a decrease in the secretion of ghrelin, which has the effect of decreasing appetite [54]. In the situation of over-nutrition, however, increased energy harvest and decreased transit time through the colon can lead to weight gain.

Peroxisome proliferator-activator receptor-γ (PPARγ) is highly expressed in the colon and can become activated by SCFAs of microbial origin resulting in an increased secretion of angiopoietin-like-4 (ANGPTL4), a circulating inhibitor of lipoprotein lipase (LPL). The result is inhibition of cellular fatty acid (FA) uptake, decreased adipose

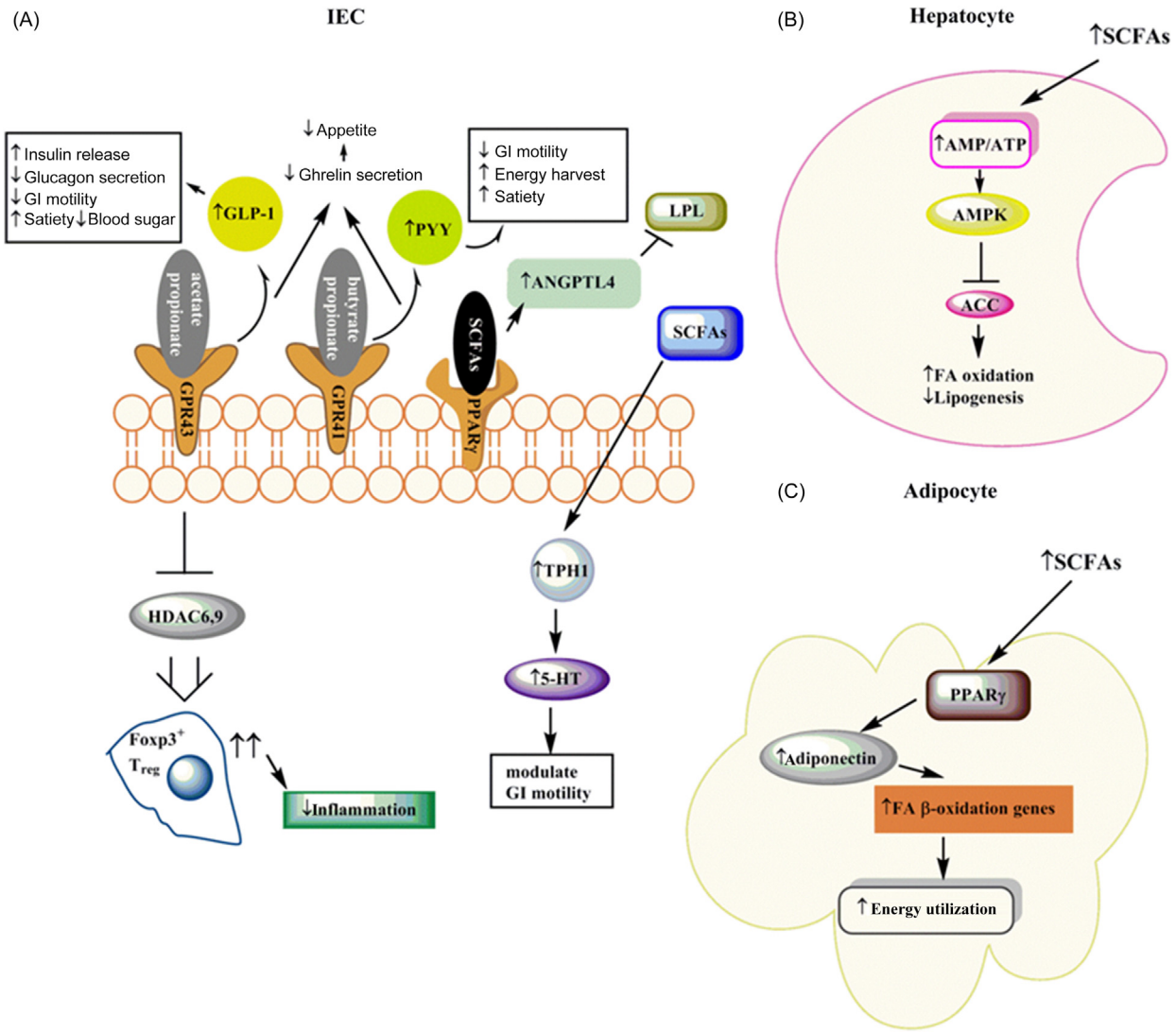

FIGURE 38.3 (A) The effect of SCFAs in IEC, (B) liver, and (C) WAT (see text for details). Abbreviations: HDAC, histone deacetylase; ACC, acetyl-CoA decarboxylase.

triglyceride accumulation, and less fat storage [55]. Confirmation for the effect of ANGPTL4 on lipid storage has come from studies of GF ANGPTL4$^{-/-}$ mice which showed the same degree of adiposity as wild-type (WT) controls [51]. Microbial SCFAs can also directly interact with IECs to cause increased expression of TRP hydroxylase-1 (TPH1), the rate-limiting enzyme for 5-HT synthesis. This means increased synthesis of 5-HT in the gut as a result of the microbiota (Fig. 38.3A). 5-HT modulates GI motility and affects energy harvest which, if stored, leads to weight gain [56].

Microbial SCFAs also impact the liver by increasing the hepatic ratio AMP/ATP. This causes increased activation of AMP-activated protein kinase and therefore, blocks acetyl-CoA carboxylase causing increased expression of enzymes for FA β-oxidation. The result is decreased de novo hepatic lipogenesis and increased energy utilization [57]. This effect affords protection against liver steatosis (Fig. 38.3B).

In the adipocyte, SCFAs can activate PPARγ in white adipose tissue (WAT) to cause upregulation of adiponectin which, in turn, increases expression of FA β-oxidation genes thereby increasing energy utilization and preventing triglyceride accumulation in WAT [58] (Fig. 38.3C). To summarize, SFCA production by gut microbiota modulates host metabolism by increasing energy harvest, decreasing blood sugar, increasing energy utilization, decreasing appetite, and either increasing or decreasing lipid storage. Over-nutrition in combination with increased energy harvest may cause weight gain and these changes may be directly related to shifts in the patterns of gut microbial communities that alter SCFA production.

A recent study of 4 female human twin pairs discordant for obesity involved fecal sampling from each twin and subsequent transplantation of the fecal samples into 8–9 week old male GF mice. Mice were then fed sterilized chow that was low in fat (4% by weight) and high in plant polysaccharides. Fecal pellets from each mouse were obtained at various time points postcolonization which were then analyzed for microbiota composition. The results showed that obesity and lean phenotypes could be transmitted to GF mice from human microbiota. Cohousing of mice given lean versus obese twin's fecal content prevented the obese phenotype. Analysis via 16S rRNA techniques revealed that in the case of cohousing there was invasion of microbiota from the lean mice into the obese but not vice versa. The most prominent invaders from lean inoculate mice to obese inoculate mice that prevented obesity were *B. cellulosilyticus*, *B. uniformis*, *B. vulgates*, *B. thetaaiotaomicron*, and *B. caccae* as well as, *Alistipes putredinis*, and *Parabacteroides merdae*. This study of humanized mice provided some strong evidence that the gut microbiota composition can directly influence adiposity [59].

In other research, genome sequencing of 16S rRNA from obese, lean, and WT mice fed the same polysaccharide-rich diet revealed that obese mice had a 50% reduction in Bacteriodetes and a proportional increase in Firmicutes compared to lean mice. Similar results were observed in humans further suggesting that the Bacteriodetes/Firmicutes ratio approached the lean phenotype after 52 weeks of induced weight loss [60]. These results have been replicated, but differences between the two phyla for obese versus lean individuals were not observed [61,62]. This led to examination of lower taxonomic levels such as genera and even specific species to define individual organisms that could be associated with obesity. One example of this is a study that showed *L. casei* and *L. plantarum* to be associated with weight loss but *L. reuteri* was associated with obesity in humans [63]. The *Lactobacillus* strain dependent effect on weight loss has brought into question the efficacy of using common probiotics in management of obesity and their therapeutic use for obesity has not to date been recommended [64]. However, the use of prebiotics has shown some promise in assisting individuals with weight loss [65]. In a study of 10 healthy volunteers of normal body mass, the effects of prebiotics (Orafti Synergy-1 from Beneo-Orafti, Belgium) on satiety and related gut-derived hormones were determined after 2 weeks of treatment. The results were increased gut microbiota fermentation, improved satiety, glucose tolerance test results, and increased plasma GLP-1 and PYY concentrations [66].

Gut dysbiosis has also been associated with the development of low-grade inflammation in obese individuals and progression from obesity to MS and IR [52]. One possible culprit for the origin of obesity-related inflammation is lipopolysaccharide (LPS), a membrane component of gram-negative bacteria. LPS is constantly produced in the gut through lysis of gram-negative bacteria and is recognized by TLR4 pattern recognition receptors in both macrophages and epithelial cells. Binding of LPS to TLRs initiates a proinflammatory cascade of events (Fig. 38.4) [67]. LPS transport from the intestinal lumen to TLR receptors has been shown to be facilitated by IEC synthesized chylomicrons after HFD feeding. Mice fed a HFD for 2 weeks showed significant increases in plasma LPS. Continuous subcutaneous low-rate

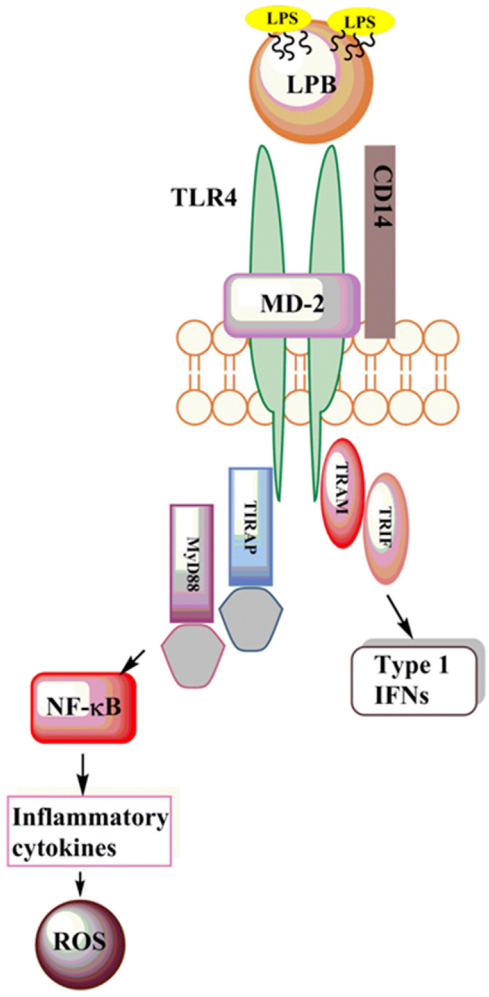

FIGURE 38.4 TLR4 activation. LPS binds to LPB and then to the CD14 accessory protein. LPS is then transferred to the next TLR4 accessory molecule, MD-2. Two TLR4/MD-2/LPS complexes must then dimerize to initiate the internal cell signaling pathways. There are two pathways for TLR4, MyD88 dependent and MyD88 independent, which lead to production of inflammatory cytokines/ROS and IFNs, respectively. Abbreviations: CD, cluster of differentiation; LPB, liposaccharide-binding protein; MD-2, myeloid differentiation factor; MyD88, myeloid differentiating primary response gene 88; TIRAP, toll-interleukin 1 receptor domain containing adaptor protein; TRIF, TIR-domain-containing adaptor-inducing interferon-B; TRAM, toll-like receptor adaptor molecule 2.

infusion of LPS into mice was sufficient to cause the HFD phenotype, that is, excessive weight gain, hyperglycemia, steatosis, WAT macrophage infiltration, and IR, thus demonstrating a link between HFD, elevated LPS, and development of MS [68]. In order to demonstrate a definite link between the TLR4 receptor for LPS and the development of obesity and MS, CD14 (Fig. 38.4) knockout mice and also TLR4-deficient mice were found to be protected from HFD-induced obesity and MS [68]. Notably, plasma LPS levels have been reported to be higher in humans with obesity and T2D than in normal controls [69]. Major LPS-producing bacteria within the phylum Proteobacteria such as Enterobacteriaceae and Desulfovibrionaceae have been found to be enriched in obese humans [65].

Does diet have any influence over activated TLR4-induced inflammation? One way in which to reduce levels of LPS is to control the population of T_H17 cells (Fig. 38.1; Table 38.2) by increasing the population of Foxp3 T_{reg} cells (Figs. 38.1 and 38.4A). Activation of GPR43 receptors by microbially derived SCFAs causes inhibition of HDAC6 and 9 that, in turn, upregulates differentiation of Foxp3 T_{reg} cells, an immunosuppressive response against the bacteria-killing T_H17 cells (reduce LPS).

Another source of obesity-related inflammation comes from TRP metabolism both by host and microbiota (Fig. 38.2). HFD and overfeeding in general can cause increased plasma concentrations of TRP from the metabolism of protein and yet morbidly obese individuals have low plasma TRP [70]. This is because 95% of TRP is metabolized along the TRP → KYN pathway and results in the increased production of substances such as COX-2 and 5-LOX which are precursors for PGEs and leukotrienes that are synthetic precursors for PICs, IFNs, and TNFα. Increased production of IFNs and TNFα cause upregulation of IDO (Fig. 38.2B) thus creating a cycle of chronic inflammation. The plasma KYN/TRP ratio is an indicator of IDO enzyme activity. TRP depletion results as IDO activity increases and this leads to less availability for 5-HT production. CNS 5-HT regulates carbohydrate and fat intake, relieves stress (caloric intake trigger) and inhibits neuropeptide Y (NYP), a potent orexigenic peptide in the hypothalamus [70]. Therefore, decreased CNS 5-HT contributes to both development and maintenance of obesity. Decreased CNS 5-HT also means less BAT further compounding the obesity situation (Fig. 38.2A) [71]. There is also lower production of melatonin, a substance important for sleep cycles. Sleep deprivation upregulates orexin activity causing NYP activation and hunger [30].

Increased peripheral 5-HT, generated from microbial metabolism in the gut (Fig. 38.2A), can activate 5-HT receptors 2B and 3A in the pancreas to cause increased glucose-stimulated insulin secretion and increased β-cell proliferation. If this situation persists, the protein translation overload associated with cellular proliferation in the pancreas can lead to increased ER stress and result ultimately in β-cell loss and IR (Fig. 38.2A) [15,60]. This will translate to development of MS and T2D. Increased IR can also be a product of the TRP → KYN pathway via the formation of xanthurenic acid (Fig. 38.2B) which is known to form a "pseudoinsulin" by reacting with insulin to form a product that is antigenetically indistinguishable from insulin but inactive, thus decreasing insulin sensitivity [30].

An important question is whether the inflammatory status can be switched back to a normal homeostasis that existed prior to the onset of obesity. It was found in one study that for postbariatric surgery patients, TRP depletion could not be prevented despite significant loss of weight and that there continued to be diminished 5-HT functions leading to unchanged satiety dysregulation and a reward-deficiency-syndrome [72]. Perhaps, there is a point of no return for chronic inflammation alleviation in the morbidly obese or perhaps the cure cannot come from bariatric surgery alone but must involve a remodeling of the gut microbiota to restore normal TRP metabolism and decreased populations of LPS-producing bacteria. Consistent with previous discussions, obesity is thus strongly influenced by the microbiota via TRP metabolism, modulation of the immune system to control inflammation, microbiota metabolic products such as SCFAs as well as compositional differences in the microbiota populations themselves.

F Gut Microbiota and IBS

IBS is one of the most common GI disorders and it is characterized by chronic abdominal pain, bloating, and abnormal defecation. Direct evidence for microbiota involvement in IBS comes from epidemiology studies which revealed that 6−17% of patients with IBS-D report an acute onset following enteric infection with *Salmonella enteridis*, *Camphylobacter jejuni,* or *Shigella flexneri* [73]. Furthermore, the use of probiotics incorporating *Bifidobacterium* spp. and *Lactobacillus* spp. and nonabsorbable antibiotics such as neomycin or rifaximin can ameliorate some of the symptoms of IBS such as bowel habits and visceral pain [74,75]. Relative to normal controls, at the phylum level, a consistent finding for IBS-D has been reported to be enrichment of Firmicutes and reduced abundance of Bacteroidetes with the Firmicutes/Bacteroidetes ratio twofold higher for IBS patients [76] along with overall lower microbiota diversity [77,78]. IBS-C, on the other hand, presents with higher levels of *Methanobrevibactor smithii*, a prolific methane-producing bacterium. Enteric methane slows intestinal

transit time and also produces ileal contractions that are nonpropulsive in nature [79]. Hydrogen sulfide, produced by sulfate-reducing bacteria, also exerts a relaxation effect on intestinal smooth muscle through an agonist action on a potassium channel resulting in decreased transit times and constipation [80].

IBS is considered a stress-related disorder and dysregulation of the gut-brain axis has been proposed as a suitable model for this disease [74]. The brain communicates with the gut via the autonomic nervous system which includes the vagus nerves (VNs), sacral parasympathetic pelvic nerves, and the sympathetic splanchnic nerves (SNs) (Fig. 38.5). The VNs transmit information to the CNS regarding luminal osmolarity, carbohydrate levels, mechanical distortion of the mucosa, and the presence of bacterial products. The afferent SNs transmit visceral pain. In addition, there are organs outside the blood brain barrier such as the adrenal gland, hypothalamus, area postrema, and the pituitary gland that respond to vascular contents of circulating cytokines and interleukins [81]. Fig. 38.5A is a diagram of the brain-gut axis. The response of the GI tract to stress can include modifications of motility, secretion, visceral sensitivity, mucosa permeability, and inflammatory responses. Mast cells (MCs) of the intestinal mucosa release PICs (Fig. 38.5A) in response to stress and induce mucosal barrier

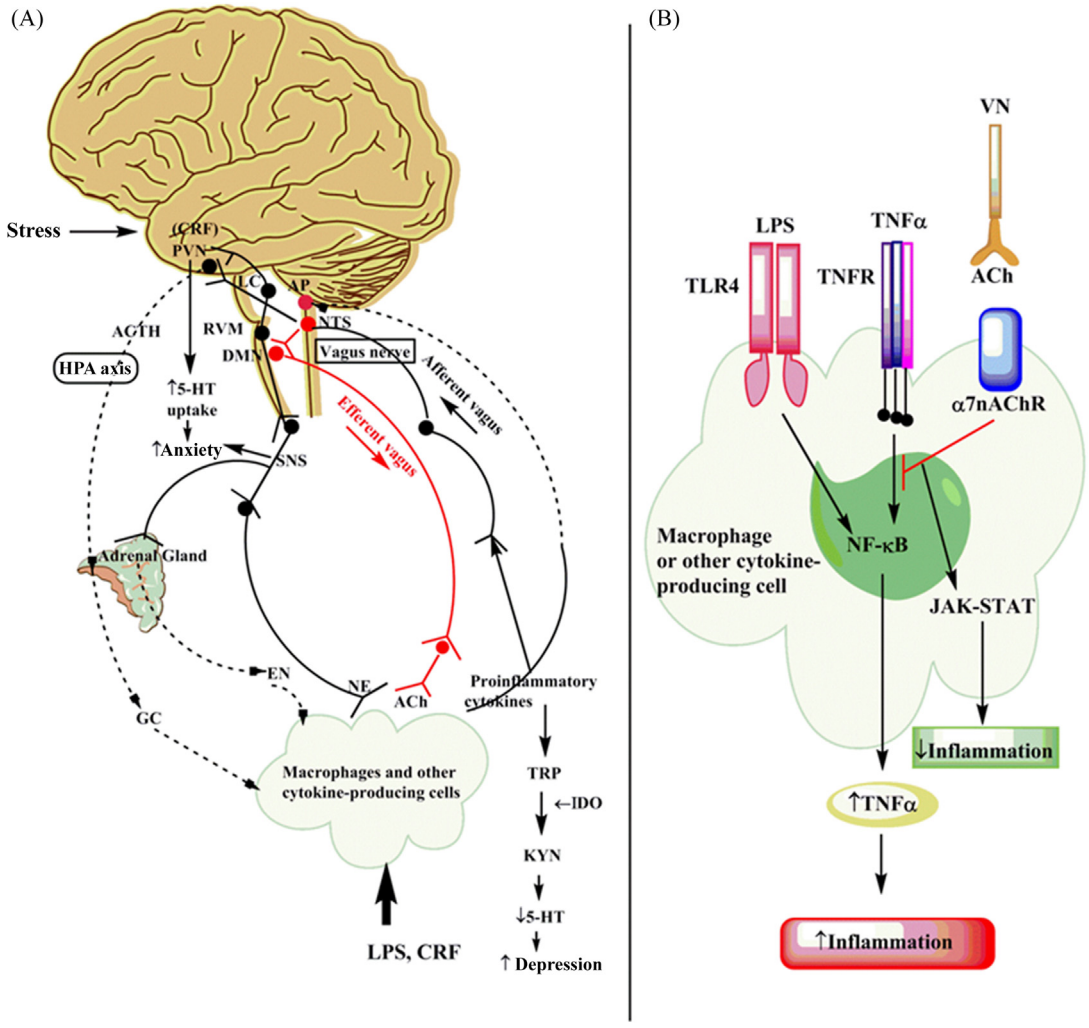

FIGURE 38.5 (A) The gut-brain axis. (B) The antiinflammatory effect of the VN via the cholinergic pathway. ACh is released at the distal end of VN efferent fibers which activates the α7nAChR receptor in macrophages. The activation of α7nAChR causes inhibition of TNFR receptor activation by TNFα thus preventing downstream activation of NF-κB signaling. Additionally, activation of α7nAChR causes activation of a JAK-STAT mediated antiinflammatory pathway [81]. Abbreviations: TNFR, tumor necrosis factor receptor; CRF, corticotrophin-releasing factor; JAK, Janus kinase; STAT, signal transducer and activator of transcription. ACTH, adrenocorticotropic hormone; DMN, dorsal motor nucleus of the vagus (origin of VN efferents); EN, epinephrine; LC, locus ceruleus (the primary brain noradrenergic nucleus located in the pons and involved in the stress response); NE, norepinephrine; PVN, paraventricular nucleus of the hypothalamus (source of corticotrophin-releasing factor, CRF); RVM, rostral ventrolateral medulla; NTS, nucleus tractus solitarii (termination point for vagus efferents).

hyperpermeability along with further activation of mucosal immunity (Fig. 38.1). This, in turn, can lead to loss of microbiota and increased amounts of bacterial products such as LPS [82]. MCs are in close contact with SNs and these afferent nerves respond to MC products via adrenergic receptor activation causing stress-induced increases in both peripheral and central PICs. This ultimately activates the NF-κB signaling pathway leading to the continued maintenance of the inflammation [83].

The VNs, on the other hand, are antiinflammatory and their activation by PICs such as IL-1β, IL-6, and TNFα results in activation of the HPA axis which, in turn, stimulates the release of glucocorticoids to reduce mucosal inflammation (Fig. 38.5A) [84]. Acetylcholine (ACh), which is released by VN efferent fibers, can decrease the macrophage production of PICs such as TNFα via activation of the α7nAChR in macrophages (Fig. 38.5B) [85]. Stress decreases VN efferent outflow of ACh and therefore favors SN-induced inflammation [86].

The gut-brain axis thus responds to stress by attempting to maintain a balance between PIC activation of VNs and SNs to produce either ACh or CRF which suppresses inflammation or increased amounts of PICs to amplify inflammation, respectively. If this balance is perturbed, IBS may result. Relative to normal patients, IBS patients have higher plasma levels of PICs such as IL-6, IL-1β, and TNFα, providing support for an inflammatory status in IBS. It has also been demonstrated that there are increased numbers of MCs in the colonic mucosa in IBS patients indicating increased activation of mucosal innate immunity and furthermore, number of MCs correlated with severity of fatigue and depression, clearly implicating MC immunomodulation of the gut-brain axis (Fig. 38.5A). Further evidence for innate immune activation in IBS comes from the observation of increased AMPs, such as β-defensin-2, secreted by IECs in response to PICs. Increased density of mucosal T-lymphocytes has also been commonly observed for IBS, implicating increased activation of mucosal adaptive immunity which can be impacted by the microbiota (Fig. 38.1) [87]. Additionally, altered TLR expression has been reported in IBS patients with specific increases in LPS-activated TLR4 (Fig. 38.5B), TLR2, and TLR5 (latter two respond to bacterial lipoproteins, flagellin, and peptidoglycans) and decreases in TLR7 and TLR8 (virus, DNA, RNA recognition). Microbial activation of TLRs 2, 4, and 5 all lead to activation of proinflammatory NF-κB signaling (Fig. 38.4) [75]. Enhanced activation of NF-κB also leads to inhibition of FXR in the intestine and the liver leading to decreased enterohepatic circulation which can lead to small intestine bacterial overgrowth a condition commonly reported for IBS-D [88].

5-HT is widely distributed in the gut and is stored mainly in ECs. IBS-D patients have been shown to release higher amounts of 5-HT postprandial relative to normal controls with the reverse being true for IBS-C patients. 5-HT release causes increased GI secretions and propulsion and if pronounced, diarrhea. Inflammation is associated with increased amounts of CD4 + T_H17 and T_H1 lymphocytes (Fig. 38.1) and it has been shown in both animal models and for human IBS-D patients that increased amounts of these cells cause EC hyperproliferation [89,90]. In addition, bacterial SCFAs can stimulate the IECs to upregulate TPH1, the rate-limiting enzyme for EC 5-HT synthesis (Figs. 38.2 and 38.4A). The SCFAs acetate and propionate have been found to be significantly increased in IBS patients of all three classifications relative to normal controls. Higher levels of acetate and propionate were correlated with increased GI symptoms, decreased quality of life, and increased negative emotions [91]. This has led to recommendations for limiting the use of prebiotics and restriction of fermentable carbohydrates (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; FODMAP diet) for IBS patients [74]. Thus intestinal transit time can be linked to the availability of TRP (from diet, microbiota proteolysis, and direct synthesis) (Fig. 38.2) as well as microbiota-directed mucosal immunity (Fig. 38.1) and SCFA production (Fig. 38.3) for increased production of 5-HT.

The effects of altered 5-HT signaling on IBS symptoms via the CNS include increased reaction to visceral pain via 5-HT activation of 5-HT_3 receptors, an increased stress response due to enhanced CRF-induced 5-HT activation of upregulated 5-HT2A receptors (Fig. 38.5A; Table 38.3) which causes increased anxiety. The combination of this increased 5-HT turnover and metabolism along with enhanced CRF-induced production of cytokines (Fig. 38.5A) can cause increased stimulation of the TRP/KYN pathway (Fig. 38.2B) eventually culminating in 5-HT depletion and depression in chronically inflamed IBS patients [92]. Clinical evidence for this comes indirectly from the utilization of the antibiotic minocycline to modulate depression [93]. Notably, minocycline has an effect of indirectly inhibiting IDO activity without affecting 5-HT turnover [94].

When TRP is metabolized to KYN in the CNS, it can be further metabolized to quinolinic acid, an NMDA receptor agonist that acts to increase excitotoxicity at glutaminergic synapses in the striatum and hippocampus where NMDA receptors are the most abundant. The result is diminished cognitive processes such as learning and memory (Fig. 38.2B) [95]. Quinolinic acid can also upregulate iNOS and this ultimately leads to increased amounts of proinflammatory PGEs and leukotrienes which can continue to fuel GI inflammation as well as sustain production of more KYN [81]. KYN can also be metabolized to kynurenic acid which is an antagonist to the α7-nAChR. CNS antagonism of this receptor results

in decreased cognition and increased depression (Fig. 38.2B) [30]. Peripheral antagonism of α7-nAChR interferes with VN release of ACh and this, in turn, can increase activation of NF-κB to favor inflammation in IBS (Fig. 38.5B) [81]. Therefore, the microbiota, via its control of TRP and SCFA availability may have a profound effect on IBS psychological symptoms and contributes to the microbiota-gut-brain dysregulation associated with IBS. Although clinical evidence is limited, one study showed that in healthy volunteers administered a probiotic consisting of *L. helveticus*, a homofermentative species that produces only lactic acid, and *B. longum*, also a lactic acid producing bacteria that has also been shown to be antiinflammatory, produced alleviation of psychological distress including an index of depression [28,96].

From the preceding discussion of IBS, it is clear that a major way to prevent/control this disease is to ameliorate stress whenever possible. Stress is the primary trigger for release of CRF (Fig. 38.5A) and activation of the HPA pathway. If the individual cannot compensate for HPA activation with VN stimulated responses, then it results in the dysregulation of the gut-brain axis. Therefore stress-reducing changes in lifestyle including changes in diet and physical activity may be able to help these patients via effects on their microbiota. More work is needed to characterize changes in microbiota that are associated with treatments that have been shown to be helpful in IBS, such as minocycline antibiotics, prebiotics, and selective serotonin reuptake inhibitors. Changes in gut microbial profiles before and after treatment may provide clues for further therapeutic targets such as identification of microbial species that are restorative of the mucosal barrier, can aid in controlling GI inflammation, and thus shift the balance of the microbiota-gut-brain axis to a balanced, noninflammatory status.

G The Gut Microbiota and ARNDD

Aging is often accompanied by regression of key physiological functions that enhance susceptibility to inflammatory diseases. "Inflammageing" is a chronic low-grade inflammatory status caused by age-related decline in the immune system, "immunosenescence" [97], thought to begin at an earlier time-point in the GI relative to other systemic compartments [98]. In the intestine, the immune response originates from gut-associated lymphoid tissue (GALT) that contain all of the cells necessary to effect antigen-specific B- and T-cell responses. After induction, immune cells migrate to the "effector site" in the LP (Fig. 38.1) where they undergo further differentiation before migration to other sites [99]. Specialized GALTs known as Paneth cells in the small intestine are the major source of AMPs and both their number and secretion functions decline with age [99].

The LP is separated from the luminal environment by a single layer of IECs which form part of the mucosal barrier via their tight junctions (TJs). In a study done in primates, aging in the absence of any overt inflammation was shown to cause increased IEC production of proinflammatory IL-6, TNFα, and IL-1β cytokines which in turn, caused aberrant TJ protein expression [100]. IL-6 has been shown to decrease murine zona occluding-1 (ZO-1) expression [101]. IL-1β has been shown to downregulate occludin in Caco-2 IECs and decrease transepithelial resistance [102]. Increased IL-6 and IL-1β have been detected in the plasma in selected healthy (65−72 years) elderly humans [103]. An upregulation of a microRNA, miR-29a, was observed in the primate study and was related to decreased expression of ZO-1, occludin, and junctional adhesion molecule-A (JAM-A) which together function in the adherence of TJ [100]. Fig. 38.6A is a diagram of the epithelial cell TJ and Fig. 38.6B shows a proposed mechanism for the effect of cytokines on TJ [104,105]. A preinflammatory state of "leaky" mucosal barrier thus opens up the possibility of increased barrier translocation of bacteria and bacterial products to eventually induce overt inflammation (Fig. 38.1) and also affect the microbiota composition.

In the brain, inflammageing manifests itself by constitutive activation of perivascular macrophages as well as parenchymal microglia that continually express PICs. This is accompanied by an increased number of astrocytes which have been shown to be capable of secreting the neurotransmitter, glutamate (GLU), an important ligand for activation of the NMDA receptor [106]. Neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's (HD) are all characterized by altered GLU homeostasis and increased excitotoxicity involving the agonist action of quinolinic acid, a product of TRP metabolism, on the NMDA receptor in brain [107,108]. Enhanced production of PICs in the brain is accompanied by increased production of reactive oxygen and nitrogen species that leads to neurodegeneration and eventually loss of neurons via KYN metabolism to 3-hydroxykynurenine (3-HK) (Fig. 38.2B) [30]. Fig. 38.7 illustrates the development of neuroinflammation from a peripheral inflammatory response [109].

The microbiota in people >65 years has been characterized by increased amounts of opportunistic aerobes such as *Staphylococcus*, *Streptococcus*, and Enterobacteriaceae, potentially pathogenic species associated with hospitalization and antibiotic treatments [110] and also decreased amounts of *Clostridium* cluster IV, XIVA, known for SCFA production, maintaining colonic pH [111], and *Bifidobacterium* (vitamin synthesis, support immune function, epigenetic effects to control cell proliferation) [111]. Aging of the gut

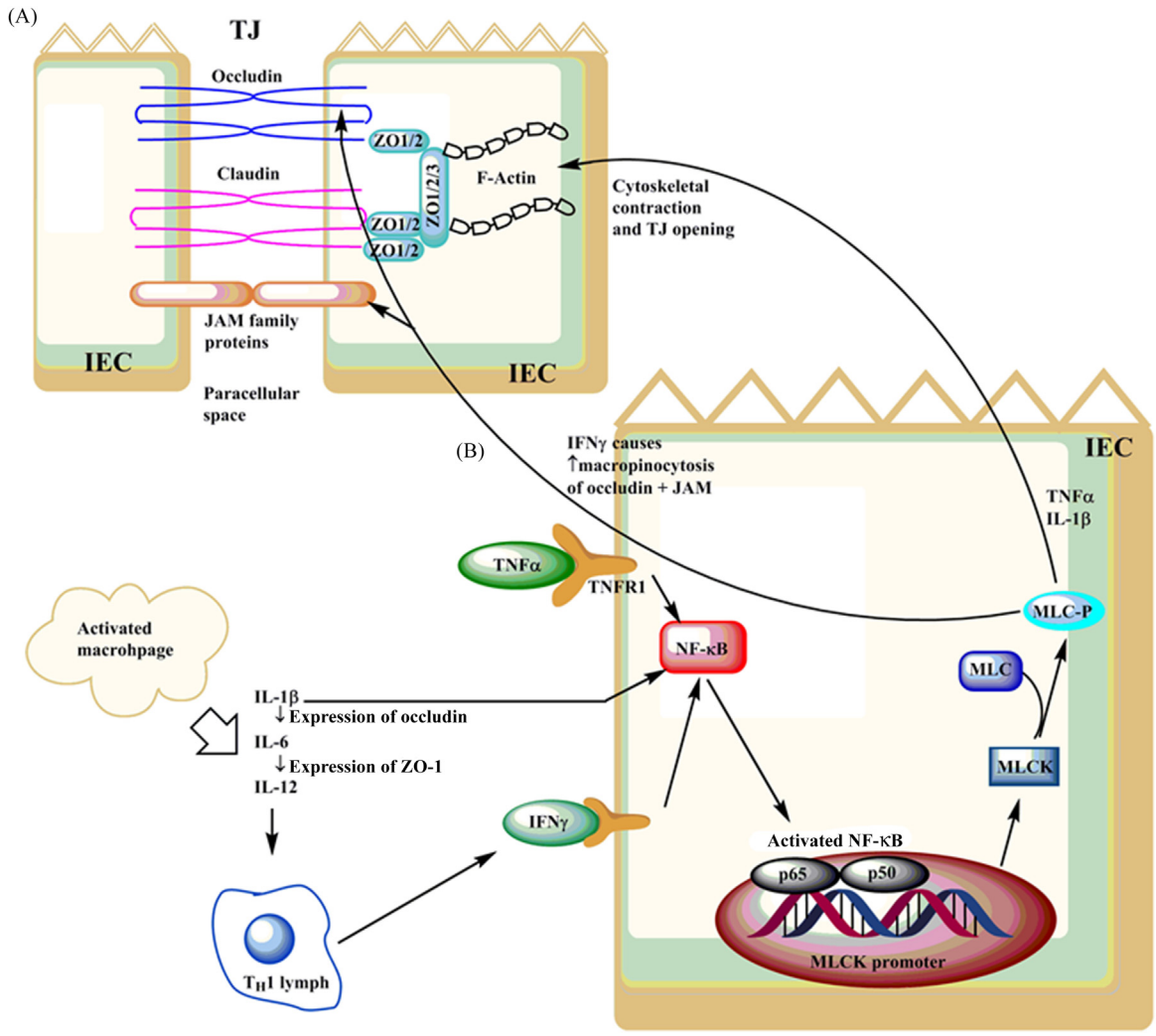

FIGURE 38.6 (A) Structure of the TJ between adjacent IECs. Transmembrane proteins occludin, claudin, and JAM family proteins seal the paracellular space between IECs. Plaque proteins such as ZO family proteins bind to one another to form a stabilizing scaffold and a direct link to the cytoskeleton via ZO-1 binding to F-actin. (B) The effect of PICs that have been found to be increased in healthy, normal weight seniors on the TJ. Activated macrophages (Fig. 38.1) produce TNFα, IL-1β, and IL-6 as well as IL-12. IL-12 goes on to stimulate production of T_H1 cells that secrete IFNγ. TNFα binds to the TNFR1 receptor on IECs and causes activation of NF-κB and its subsequent translocation to the nucleus where it acts as a transcription factor for the MLCK promoter resulting in production of MLCK protein. MLCK then phosphorylates MLC to MLC-P which then acts on F-actin to cause cytoskeletal contraction and opening of the TJ. IL-1β has been shown to downregulate the expression of occludin and also acts via NF-κB → MLC-P to cause TJ opening. IL-6 has been shown to decrease expression of ZO-1 causing disruption of the TJ stabilizing scaffold. IFNγ, also via MLC-P, has been shown to increase IEC macropinocytosis of occludin and JAM proteins, thus removing them from the paracellular space. The culmination of all of these effects is a "leaky" mucosal membrane. Abbreviations: MLCK, myosin light chain kinase; MLC, myosin light chain protein; TNFR1, tumor necrosis factor receptor-1.

microbiota has also been shown to exhibit diminished bacterial diversity with increased amounts of proteolytic bacteria and decreased amounts of saccharolytic bacteria [36,112]. Increased proteolytic bacteria allow for more TRP production and decreased populations of saccharolytic bacteria implies less production of SCFAs (Figs. 38.2 and 38.5A). The former leads to activation of proinflammatory pathways (Fig. 38.2) and the latter can adversely affect IEC health and augment inflammation via diminished effects on T_{reg} cell populations (Fig. 38.1).

Investigators have started to examine changes in gut microbiota in PD and AD patients. In a study of 72 subjects and age-matched controls, PD subjects showed higher counts of Enterobacteriaceae and a 77% reduction in Prevotellaceae, relative to controls. The latter is known to be saccharolytic, providing SCFAs as well as, thiamine and folate. Decreased amounts of thiamine and folate could potentially result in decreased production of vitamins necessary for important gut hormone secretion [113,114]. Certain cyanobacteria normally present in small

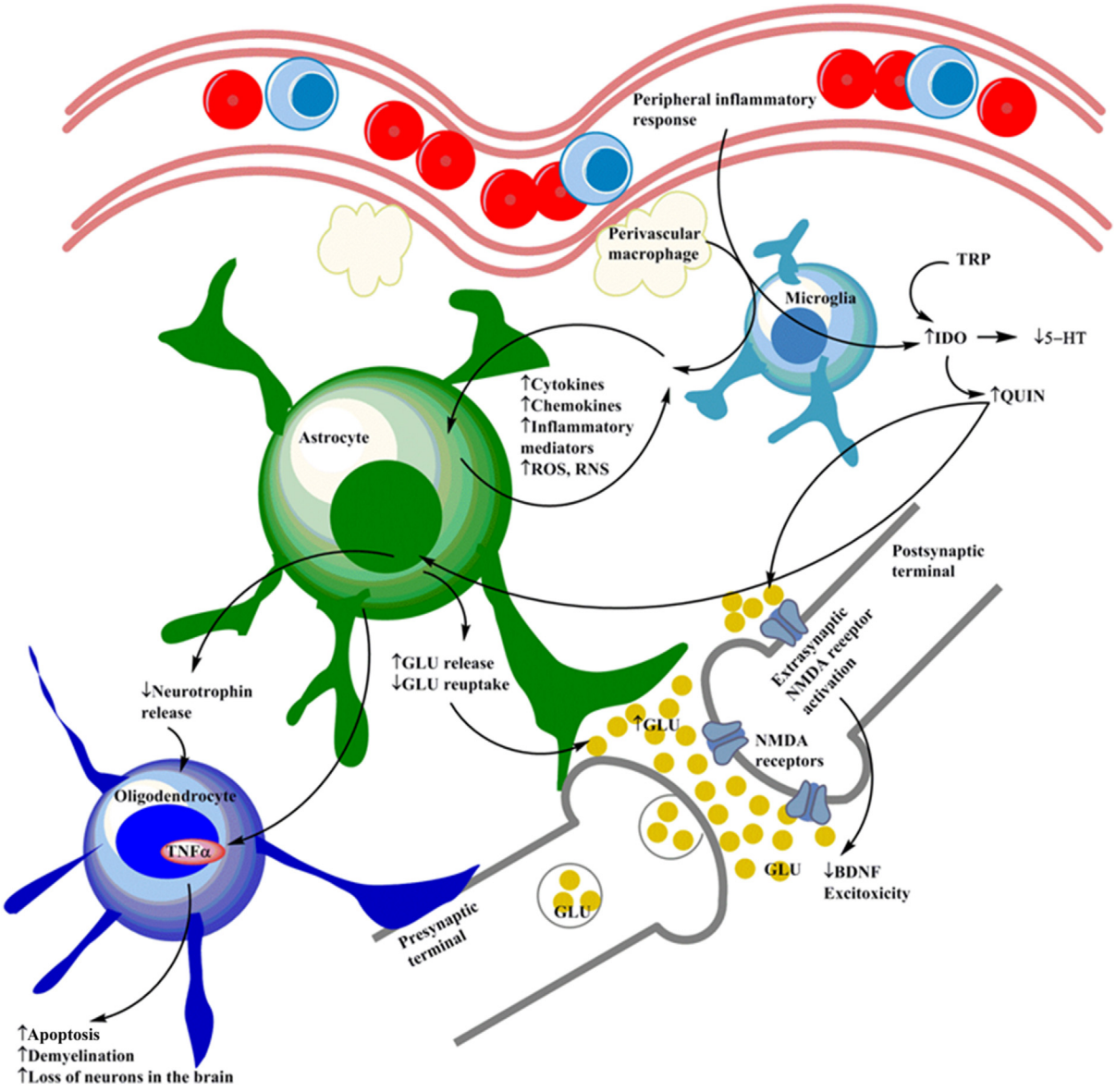

FIGURE 38.7 Inflammageing in the brain. An inflammatory response initiated in the GI can result in circulatory transport of proinflammatory substances such as LPS which can activate perivascular macrophages and microglia to upregulate IDO and the TRP/KYN pathways resulting in increased production of neural PICs, ROS, and RNS. Increased TRP metabolism causes decreased levels of 5-HT and increased levels of QUIN. QUIN is an agonist for the excitatory NMDA receptor and thus stimulates release of glutamate (GLU). Multiple functions of astrocytes are compromised due to excessive cytokine exposure resulting in a decrease of GLU reuptake and an increased release of GLU along with decreased trophic support for oligodendrocytes. Oligodendrocytes are very sensitive to PICs, especially TNFα. Exposure to excess TNFα leads to oligodendrocyte apoptosis and eventual loss of neurons in the brain. Overall, inflammageing causes a disruption of neural plasticity through glutaminergic excitotoxicity involving the NMDA receptor with subsequent degeneration and apoptosis of neurons.

numbers in the GI tract produce β-*N*-methylamino-L-alanine (BMAA) which has been found to be elevated in the brains of AD and PD patients. BMAA is an excitotoxin that activates metabotropic glutamate receptor 5 causing depletion of the antioxidant, glutathione, and defective glial cell control of neuronal ROS and RNS. BMAA has also been implicated in the protein misfolding and aggregation seen in PD and AD patient brains [114,115].

Bacteria also have the capacity to generate neurotransmitters. Certain *Lactobacillus* sp. and *Bifidobacterium* sp. produce gamma-aminobutyric acid (GABA); *Escherichia*, *Bacillus*, and *Streptococcus* spp. produce NE; *Candida*, *Streptococcus*, *Escherichia*, and *Enterococcus* spp. produce 5-HT; *Bacillus* produces dopamine (DA); and *Lactobacillus* can generate ACh [116]. It should be noted here that GABA-producing strains of *L. helveticus R0052*

and *B. longum R1075* in a probiotic formulation have shown efficacy in alleviating depression symptoms in both animal studies and human volunteers [96]. GABA is significant because it is an inhibitory neuromodulator in the CNS and hence works to counter the effects of excitatory GLU signaling [117].

TRP metabolism alterations play a large role in aging. Aging has been associated with elevated cortisol production as a result of HPA axis hyperactivation (Fig. 38.5A). This hyperactivation results in enhanced production of cortisol which upregulates TDO (Fig. 38.2B). Another consequence of enhanced cortisol production is desensitization of glucocorticoid receptors which diminishes the antiinflammatory effect of glucocorticoids. Thus, the activation of not only IDO but also TDO (Fig. 38.2B) causes an increased risk for depression in the elderly via TRP metabolism to KYN and increased inflammation [118]. Evidence for age-related TRP depletion has been found in several studies, one of which revealed increased amount of genes for TRP metabolism [119]. Another study of centenarians revealed plasma TRP depletion [120]. Two independent studies of elderly patients detected increased KYN/TRP ratios indicating increased TRP metabolism to KYN relative to younger patients [121]. Neurodegenerative disorders associated with aging such as PD and AD have long been associated with depression accompanied by TRP, 5-HT, and melatonin depletion [122,108]. Additionally, HD, a neurodegenerative genetic disorder that affects early aging at 35−45 years of age has also been associated with the TRP depletion [107,123]. In fact, a point of similarity for all three of these neurodegenerative disorders is that there is a decreased amount of kynurenic acid (thought to be neuroprotective) but increased amounts of 3-hydroxykynurenine (3-HK) and quinolinic acid indicating a preferred metabolism of KYN to quinolinic acid, a known NMDA receptor agonist, which is consistent with the NMDA excitotoxicity and oxidative stress that is associated with these conditions (Fig. 38.2B) [108].

Thus, the homeostatic balance in the brain that is disrupted as a result of gut microbiota-induced inflammation that starts in an aged "leaky gut" is the balance between excitatory (GLU) and inhibitory (GABA) neurotransmitter signaling in the brain. The homeostatic shift in the balance is toward glutaminergic excitotoxicity and this leads to a progression from depression to neurodegenerative diseases. The reason for this shift is due to another shift in the balance, that is, a shift toward KYN metabolism to QUIN away from KYN metabolism to kynurenic acid. The use of probiotics that replace the diminished *Lactobacillus* and *Bifidobacterium* spp., a situation commonly found in the aged population, has shown some promise in alleviating depression perhaps by their ability to produce the neuroinhibitory neurotransmitter, GABA. The fact that neuroinflammation is the driving force in depression is evident from the fact that antioxidants such as melatonin, NSAID, and more recently, antiinflammatory antibiotics such as minocycline show efficacy in treatment [109,124,125]. Therefore, correcting the gut dysbiosis as early as possible may be the key to prevention of age-related dementias.

H The Metabolomic Approach to the Study of Microbiota

Metabolomics is a new omics approach aimed at qualitatively and quantitatively describing all of the metabolites in cells, tissues, or body fluids. The ultimate goal of an analysis is identification of a complete panel of metabolites that reflect important changes resulting from disease acquisition or progression. A targeted approach is used to examine a predefined set of metabolites. Liquid chromatography (LC) and/or gas chromatography (GC) interfaced to a mass spectrometer are the most widely used techniques, while nuclear magnetic resonance spectroscopy is growing in its use in metabolomics. The general steps in a typical metabolomics analysis include sample collection and processing, instrumental analysis including separation and detection of individual metabolites, data analysis, and interpretation [126]. GC is useful for compounds that are volatile and also thermally stable enough to survive higher than ambient temperatures. LC is employed for higher MW compounds and thermally sensitive substances. It is not uncommon for both techniques to be applied on aliquots of the same sample to broaden the metabolic profiling capacity. Some key differences in protocols for metabolomics also concern with sampling and pretreatment (i.e., body fluid vs cell lysate) as well as instrumental parameters (column selection, GC vs LC, temperature and solvent gradients, etc.). Metabolomics is a powerful tool that can be used to identify microbial metabolites, test the efficacy of a therapeutic intervention [127], reveals an underlying cause for a disease state [128,129] or to predict disease acquisition risk [130].

A specific example of the application of metabolomics to characterization of microbial activity and metabolism is the examination of metabolic impact on host metabolism in mice with their gut microbiota ablated using a broad spectrum antibiotic and measurements of their metabolic phenotype were done at multiple time-points. Zheng et al. first reported a panel of 202 urinary and 223 fecal metabolites derived from gut microbial−mammalian cometabolism, which includes TRP metabolites, SCFAs, organic acids such as hippuric acid, amino acids such as tyrosine and phenylalanine, and oligopeptides [129]. These differentially expressed metabolites were identified in a rat model treated with beta-lactam antibiotic

imipenem/cilastatin sodium for 4 days followed by a 14-day recovery period. Increased amounts of TRP, tryptamine, and melatonin along with decreased amounts of indole and other TRP metabolites such as 3-HK were observed in urine and feces. Neurotransmitters such as DA, NE, and epinephrine were also perturbed in both urine and feces. These results lend credence to the impact of gut microbiota on the gut-brain axis [129].

Another approach of identifying microbial metabolome is the direct measurement of metabolites produced by bacteria. A recent study examined the effect of different sizes of arabino-oligosaccharides (AOS) on six different human fecal communities from healthy subjects in an in vitro experiment [127]. The AOS substrates were obtained from sugar beet and were separated using HP size exclusion LC to recover the original AOS base-extract (BE), an enriched low MW component (LA) and an enriched high MW component (HA). Fecal samples from six healthy volunteers were then collected in airtight containers and fecal slurries prepared anaerobically were then mixed with BA, LA, or HA. Bacterial DNA was extracted and was measured by qPCR using 16S rRNA targeting primers. Metabolites were measured by LC-mass spectrometer. Comparisons were made between the two time-points (before and after bacterial fermentation), and to a control with no AOS added. BE and LA fermentations yielded similar results while HA was distinct in yielding higher amounts of *Bifidobacterium* spp. and *Desulfovibrio* spp. The importance of this study was not only identification of HA as a potential prebiotic but correlations made with specific metabolites thus expanding the repertoire of potentially useful bacterial metabolites other than SCFAs. For HA samples, these included increased amounts 3-oxaloalanine, tyramine, and homoveratic acid. On the other hand LA and BE had higher levels of cysteine, aminobenzoic acid, hypoxanthine, and 3-octadecanoic acid. By using metabolomics, the efficacy of a potential prebiotic therapy as well as baseline levels of potentially beneficial plant metabolites for a healthy microbiota were obtained.

II CONCLUSION

In this chapter we have presented some specific examples of the way gut microbiota influence human health and how they may contribute to the development of noncommunicable inflammatory disease such as obesity, IBS, and ARNDD. The gut microbiota modulate inflammation via their effect on immunity, their own populations, availability of neurotransmitters, key metabolites for host health, and the enterohepatic circulation via BA metabolism for GI tract homeostasis. Future therapeutic targets for noncommunicable inflammatory diseases may have to go beyond treatment of inflammation in order to control the epidemic that developed countries are now facing with respect to the topics covered in this chapter and other chronic inflammatory conditions such as cardiovascular disease and T2D. Knowledge of how to reestablish the original homeostatic condition may require a more detailed dissection of the combination of environmental and dietary factors that give rise to the gut dysbiosis now believed to be important in the etiology of most of these conditions. Only by resetting the "shift" in the homeostatic balance can these conditions be said to be truly "cured." A better understanding of the microbiota composition and its response to conventional treatments already in use may provide clues to design better pro- and prebiotic strategies to correct dysbiosis along with "prescription diets" to encourage a healthy, diverse population of commensal bacteria. A key component to this will be the application of metabolomics to evaluation of clinical improvements as a result of therapeutic intervention in an effort to provide key information linking host metabolism with changes in the gut microbiota.

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Chapter 39

Nutritional Management of Inflammatory Bowel Disease and Short Bowel Syndrome

Jennifer L. Barnes[1] and Kelly A. Tappenden[2]
[1]Illinois State University, Normal, IL, United States, [2]University of Illinois at Urbana-Champaign, Urbana, IL, United States

I INFLAMMATORY BOWEL DISEASE

Crohn's disease (CD) and ulcerative colitis (UC) are the two most common forms of inflammatory bowel disease (IBD). Although the precise etiology is not known, evidence suggests that the body launches an immune response to a healthy, normal intestinal environment, resulting in inflammation (Fig. 39.1). Inflammatory episodes range from mild to very severe and are often relapsing. Over the last several decades, the prevalence of IBD, particularly CD, has been on the rise, suggesting an environmental contribution, with evidence for a genetic component [1]. It has been suggested that individuals in Western countries including North America and Europe are more likely to develop IBD, but perhaps not coincidentally; this is also where the greatest number of population-based studies are performed. Recent estimates suggest an incidence of 19.2 UC cases per 100,000 people in North America and 20.2 cases per 100,000 people for CD [2] with a prevalence of 1.3 million IBD sufferers in North America alone [3]. Caucasians and individuals of Jewish descent show a greater incidence of IBD; females are at increased risk of CD compared to males, whereas UC is slightly more common in men [2,3].

The cause of IBD is yet to be elucidated, but it is likely due to unusually aggressive innate and adaptive immune responses. This immune response is thought to be in response to interactions with the gut microbial communities, which may be mediated by genetic predispositions [1,4,5]. The gastrointestinal (GI) tract is continually exposed to numerous antigens, presented from the diet, microbiota, and other environmental factors, each with the potential to activate an abnormal immune response, thereby initiating an IBD episode. These interactions are an active area of research, with emphasis on the possibility of a microbiota dysbiosis being of particular etiological importance.

IBD significantly impacts the patient's health, quality of life, nutritional status, and relationship with food. Nutrition is a key component of any IBD care plan. Food choices can trigger or exacerbate, and even reduce the symptoms of IBD. During active disease and following surgery, adequate nutrition is critical to prevent deficiencies. This chapter will review IBD and short bowel syndrome (SBS). Topics covered for each will include variants of the diagnosis, etiology, a brief orientation to medical management, and an in depth discussion of the medical nutrition therapy.

A Characteristics of IBD

CD and UC both result from a robust, cytokine-driven inflammation of the GI tract [6]. The prolonged and dysfunctional inflammatory response results in changes in intestinal mucosal architecture, strictures, bowel thickening, ulcerations, altered motility, and malabsorption [6–8]. Symptoms of fatigue, intestinal and extraintestinal pain, bloating, diarrhea, steatorrhea, weight loss, malnutrition, and increased risk of colon cancer are manifestations of increased local and circulating levels of cytokines and other products of inflammation [9,10]. Impaired intestinal permeability, local edema, ulceration, and damage to the intestinal structures may occur. Secretions, digestion, absorption, and motility may be abnormal, especially in

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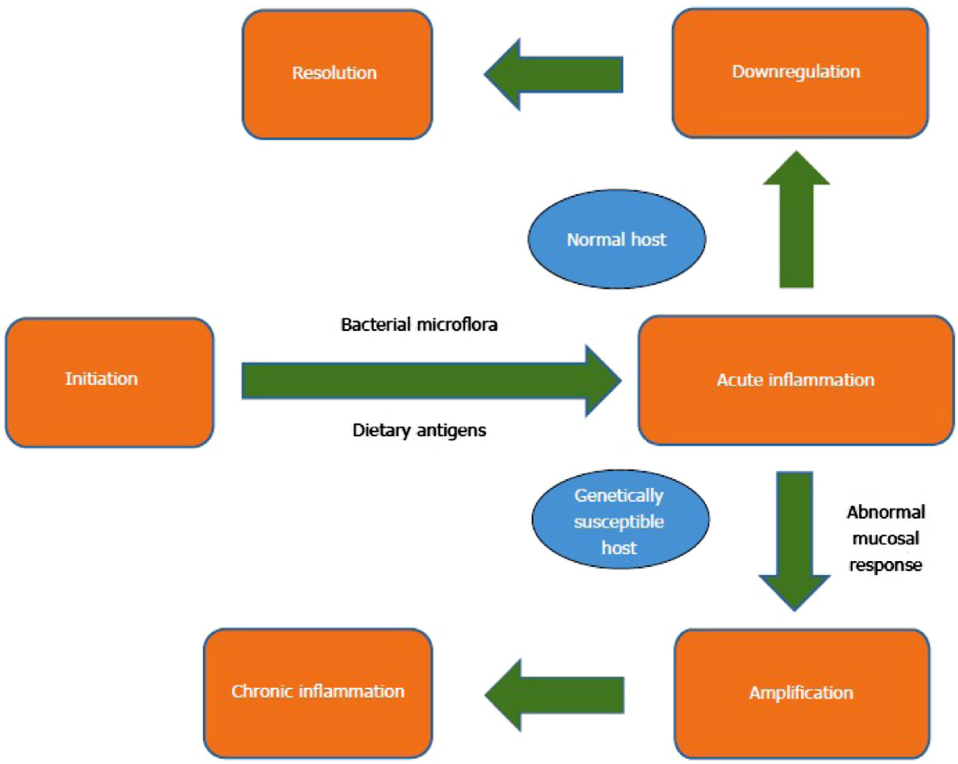

FIGURE 39.1 Summary of events leading to IBD. *Reprinted from A. Wędrychowicz, A. Zając, T. Przemysław, Advances in nutritional therapy in inflammatory bowel disease: review, World J. Gastroenterol. 22 (3) (2016) 1045–1066, with permission from Baishideng Publishing Group Inc.*

active stages, and the normal barrier function may be compromised [6,7]. Malabsorption results in macro- and micronutrient deficiencies with consequences such as anemia and metabolic bone disease. Some CD and UC patients experience arthritis and dermatologic manifestations due to the widespread inflammation. Many of these symptoms are shared by the two inflammatory conditions, but others are unique to either CD or UC.

The most important differentiating characteristic between CD and UC is that patients with CD can present with inflammation occurring through the length of the GI tract that is not limited to the mucosa. Indeed, inflammation in CD is transmural, affecting the full thickness of the tissue. The anatomical location of the disease may change over time, but is most commonly found in the distal small intestine. Specifically, at diagnosis CD is located in the ileum 47% of the time, in the colon 28% of the time, and both ileum and colon together 21% of the time [7,11]. Diseased tissue is often intermittent and surrounded by healthy tissue. Malabsorption, mucosal thickening, strictures, obstruction, abscess and fistula formation, and kidney stones are also more common with CD (see Fig. 39.2). Unlike CD that is associated with more proximal and transmural inflammation, UC is confined to the colon and inflammation affects the mucosa only. Progression of UC differs from that of CD in that it

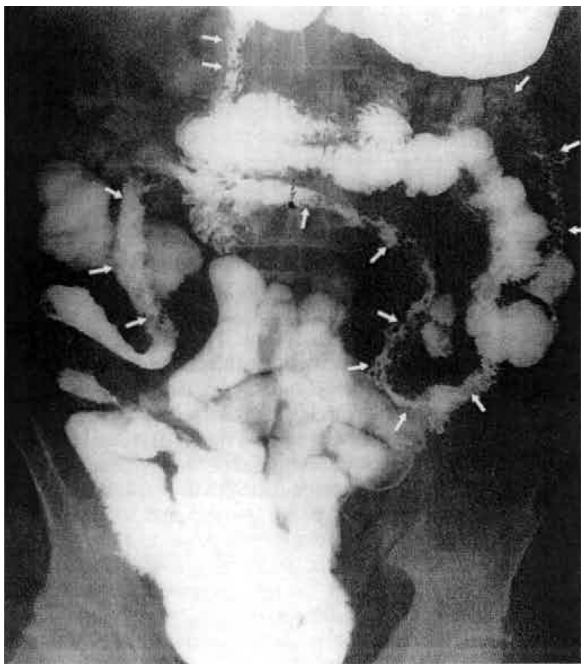

FIGURE 39.2 Radiologic image of patient with CD. White arrows indicate areas of narrowed small intestine. *Reprinted from M. Sleisenger, M. Feldman, Sleisenger and Fordtran's Gastrointestinal and Liver Disease, seventh ed., W.B. Saunders, 2002, with permission from Elsevier.*

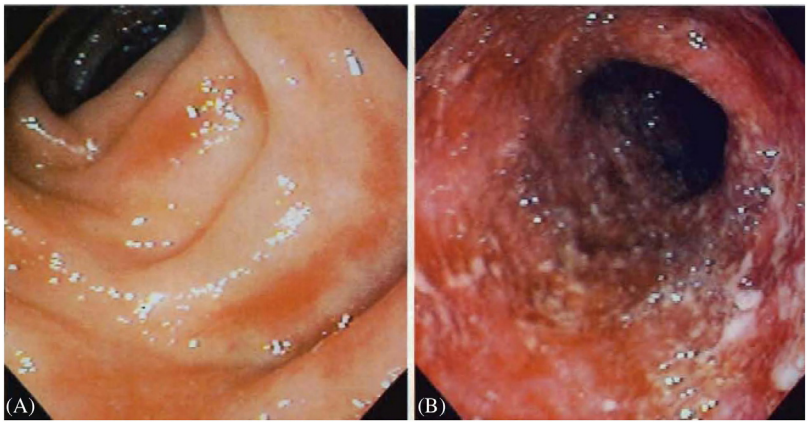

FIGURE 39.3 (A) Mild UC with some evidence of hemorrhage; (B) severe UC with extensive hemorrhage. *Reprinted from M. Sleisenger, M. Feldman, Sleisenger and Fordtran's Gastrointestinal and Liver Disease, seventh ed., W.B. Saunders, 2002, with permission from Elsevier.*

TABLE 39.1 Characteristics of CD and UC

Crohn's Disease	Ulcerative Colitis	Both
• Can occur in length of GI • Transmural • Healthy mucosa interrupted with diseased mucosa • Fistulas • Strictures • Nausea and vomiting	• Bloody diarrhea • Anemia • Limited to the colon • Mucosal ulceration	• Relapse and remission • Abdominal pain • Diarrhea • Tenesmus • Loss of appetite • Fatigue • Weight loss, growth failure • Systemic inflammation • Fever • Increased risk of colorectal cancer

characteristically begins at the most distal tissue and extends proximally in a continuous fashion. The hallmark of UC is bloody diarrhea and mucus (see Fig. 39.3) [7,8,12]. See Table 39.1 for common disease characteristics.

B Variables Triggering Onset or Exacerbation of IBD

IBD is a chronic disease characterized by relapse and remission. Environmental factors can trigger relapse or help maintain remission and knowledge of these factors are important for clinical management. However, the potential triggers are many, including genetic and environmental contributions, and often difficult to predict [13]. In addition, a number of years may elapse between histological onset, symptoms, and IBD diagnosis. Microscopic or endoscopic evidence of the disease may occur long before clinical symptoms become apparent. Normal reactions to dietary indiscretions may cause GI symptoms (gas, bloating, pain, cramping, diarrhea) and may be confused with active disease. Dietary factors considered to provoke active disease include high added sugar intake [14], specific lipids, lack of dietary fiber (fruits and vegetables) [12,15–18], abnormal metabolism of sulfur-containing amino acids as in hyperhomocysteinemia [17,19], inadequate micronutrient status such as folate [19,20] and vitamin D [21], and individual food intolerances or food allergies [13]. Breastfeeding and consumption of fruits, vegetables, dietary fiber, and *n*-3 lipids are considered protective [7,12,18,22].

C Medical Management of IBD

Several classes of medications are utilized to control complications and induce/maintain remission of IBD, but there is no cure [23]. The most common categories of medications include antiinflammatory agents, immunomodulator or immunosuppressive compounds, biologic agents, and antibiotics [23,24]. Drugs are also prescribed or recommended to treat specific symptoms, including antidiarrheals, antiemetics, analgesics, and antibiotics [23,24]. While often helpful in managing IBD, in some cases medications are not effective at inducing or maintaining remission even at high doses and for prolonged periods of time [24]. A nutrition professional must always

TABLE 39.2 Medical Management of IBD

5-Aminosalicylic acid (antiinflammatory)
• mesalamine, sulfasalazine
Corticosteroids
• prednisone, prednisolone
Immunosuppressives
• azathioprine, 6-mercaptopurine, methotrexate
Biologicals
• infiximab, adalimumab, etanercept, certolizumab
Antibiotics
• antimycobacterial, metronidazole

be aware of medications prescribed to patients to monitor for any potential drug−nutrient interactions. See Table 39.2 for a listing of medications commonly used in IBD.

D Surgical Treatment of IBD

When management of IBD through medications ceases to be adequately effective, surgical intervention is often utilized to control CD and/or cure UC. Specific indications for surgery include severe, unrelenting disease, strictures, obstruction, hemorrhage, increasing risk of cancer, repair of fistulas, and failure of other medical therapy [12,25]. As UC is restricted to the colon, a complete colectomy eliminates any further relapse and is considered curative. It is estimated that approximately 25−30% of patients with UC have surgery during their lifetime [26−28]. The most common surgical intervention is colectomy with the creation of an ileoanal pouch. This pouch develops the microbiota and serves to some degree as a colonic/rectal reservoir.

Unlike UC, surgical resection of severely involved segments of small or large bowel in CD does not bring resolution of the disease and duration of clinical remission varies greatly. In fact, many patients experience relapse which may occur in months, whereas other patients appear to remain in remission for years. Approximately 70−90% of patients with CD eventually have at least one surgery during their lifetime with 40−50% requiring additional operations [29−31]. Strictureplasty is a procedure to relieve narrowed segments of bowel generally caused by fibrous tissue. This procedure may be preferable as it helps to preserve intestinal tissue [32]. However, in some cases, patients with prolonged or severe episodes of CD have multiple resections resulting in SBS and the host of new nutritional considerations associated with that diagnosis (covered later in this chapter).

E Nutrition Assessment in IBD

Nutrition assessment in IBD should follow the standard approach including collection of anthropometrics, biochemical measures, clinical evaluation, and dietary intake data in order to fully evaluate nutrition status and risk. Standard anthropometric measurements including growth rate for age in children, weight and history of weight changes, body mass index, and body composition assessment when available are important in IBD. Biochemical laboratory data should be evaluated for protein-energy and micronutrient status. Evaluation of the patient's medical and surgical history including duration and severity of the disease, presence of strictures, fistulas, resections, and ostomies are necessary to identify nutritional risk. Symptoms including diarrhea (stool volume, frequency, and duration) or malabsorption (increased fecal fat), very low serum cholesterol, abdominal cramping, bloating, or distention also significantly affect nutritional status. A careful diet history of typical food intake, quantity and quality of food choices, and intolerance and aversions to various foods and food allergies provides insight into macro- and micronutrient intake. IBD patients may utilize botanical or other complementary medicines [33] in addition to prescriptions and over-the-counter medications, which may or may not influence the nutrition management of the disease. The nutrition assessment should also include an evaluation of the patient's knowledge and understanding of his or her nutritional status, needs, problems, and therapeutic options.

1 Energy Balance

When conducting the nutrition assessment, close consideration must be paid to energy balance and weight status as patients with IBD often experience anorexia and malabsorption as a consequence of the widespread inflammation [34−38]. Individuals may also avoid food due to symptoms and perceived food intolerances, negatively impacting the amount and quality of food consumed. Weight loss, muscle wasting, growth failure, and delayed maturation are some of the most common problems in adults and children with IBD, particularly CD. Evidence of protein-energy malnutrition may include decreased levels of transport proteins such as albumin, transferrin, or prealbumin [39]. Decreased intake is further confounded by increased fecal loss of macronutrients during IBD flares. Growth failure is common in children suffering from IBD, particularly CD [40−42]. The etiology of growth failure is similar to that of weight loss and wasting in adults where anorexia and malabsorption are the primary causes [43].

2 Fluid and Micronutrients

Diarrhea is one of the most common symptoms exhibited in IBD [7,12]. Dehydration and electrolyte imbalances may occur in flares of IBD and after significant small bowel resections in CD. Micronutrient deficiencies

secondary to malabsorption and/or inadequate intake including vitamin B_{12}, folate, zinc, calcium, iron, magnesium, selenium, copper, and vitamin A, D, and E have all been reported in IBD [39,44–47]. Further, anemia may result from lack of one or several of these micronutrients resulting from inadequate intake, malabsorption, increased requirements, and drug–nutrient interactions. As many as 70% of children and 40% of adults with IBD are anemic [44]. UC patients may be at increased risk for iron-deficiency anemia due to blood loss in feces, while CD patients are at greater risk following intestinal resection [48,49]. Overall, micronutrient deficiencies may be more profound in CD patients, especially those who have undergone surgical resection [50,51]. Depending on the location of resection and severity of malabsorption, intravenous supplementation of micronutrients, particularly iron and B_{12}, may be indicated [52,53].

F Medical Nutrition Therapy in IBD

1 Goals of Medical Nutrition Therapy

The primary nutritional goals in IBD are to restore, support, and maintain nutrition status, help control symptoms, and decrease inflammation. Inadequate intake of protein, energy, and micronutrients, independent of a diseased state, are known to effect changes in GI barrier function and digestive functions, modulate immune function, and alter microbial populations [54]. Further, individual dietary components are well-established antioxidants and may serve as precursors to inflammatory mediators in the GI tract. These may be implicated in remission maintenance [55,56]. More specifically to IBD, nutritional rehabilitation may be required after acute or prolonged reduction in the quantity or quality of dietary intake. Special diets and supplements may be recommended to provide adequate nourishment with complications such as SBS, strictures, or fistulas especially in CD. Patients may experience severe malnutrition, particularly following aggressive bouts of the disease, or after surgical procedures if adequate preoperative nutrition care and education was unavailable. Indeed, focusing on achieving optimal nutrition status prior to surgical interventions has been shown to improve patient outcomes [57]. Nutrition counseling can also aid the patient in identifying and avoiding foods that exacerbate symptoms. Intolerances are highly individualized and should be treated on a case-by-case basis. In addition, patients may choose to avoid foods they perceive as responsible for adverse symptoms such as diarrhea, abdominal pain, nausea, and bloating. Finally, medications can influence appetite and GI-related symptoms, resulting in decreased intake, increased malabsorption, and/or alter requirement for nutrients. Potential drug–nutrient interactions should be closely monitored and discussed with their health professionals.

2 Energy and Protein Requirements

There is no evidence to suggest that patients with IBD have increased energy requirements, unless nutrition status restoration or weight gain is warranted. Other complications known to increase resting energy expenditure may simultaneously occur resulting in elevated energy needs such as sepsis or fever. Regardless, the presence of active disease alone does not appear to raise energy requirements appreciably [58,59]. Protein needs may be increased significantly because of GI nitrogen losses, the ongoing/established inflammatory response, and the anabolic synthesis of new tissue for healing postoperatively, weight gain, and/or growth.

3 Parenteral Nutrition During Active IBD

In previous years, parenteral nutrition (PN) was a primary therapy in IBD, particularly CD. It was observed that patients on PN appeared to enter remission. This initiated a protocol wherein PN was provided as a means of "bowel rest" with the rationale of decreasing the antigen load presented to the mucosa [60–63]. However, the concept of bowel rest is no longer in favor. According to the 2009 European Society of Parenteral and Enteral Nutrition (ESPEN) Guidelines on PN in Gastroenterology, PN is not indicated as a primary therapy in the treatment of CD or UC [64]. PN is indicated in patients with obstruction, perforation, complicated fistula, SBS, or a nonfunctioning GI tract that precludes adequate enteral nutrition (EN). Withholding oral/EN is considered undesirable because it may compromise gut integrity including atrophy of the intestinal mucosa [65,66]. In addition, PN does not contain the balance of phytochemicals typically present in plant foods and complex diets that may interact with the inflammatory response. Further PN is being associated with greater cost and increased risk of sepsis [67].

4 EN During Active IBD

EN is the route of choice for nutrition support whenever possible. In almost all studies, CD appears to be more amenable to enteral treatment than UC, although different forms of nutrition interventions (other than standard enteral formulas) may be of value in UC. Guidelines from ESPEN [68] cite that enteral feeding is first-line therapy in CD and should be used as sole therapy in adults when treatment with steroids is not feasible. Undernourished patients with CD or UC should receive oral or tube-fed supplements.

The most recent and robust evidence-based reviews of the CD literature [68,69] indicate that medicinal corticosteroid treatment is more effective than EN for the acute phase of UC and CD. However, when considering EN, a clear superior choice among elemental and polymeric formulas is not seen [70]. Exclusive enteral feeding appears

to be more effective than partial EN, while lower fat formulas are more favorable. Remission rates in the individual studies ranged from 20% to 84%. Overall, enteral diets have the advantage of providing/restoring nutritional status and reducing the risks of medications. The majority of reports indicate that mucosal healing is more likely to occur with enteral feeding than with steroids due to a decrease in mucosal cytokine production [71,72]. The possible mechanisms for these benefits include an altered antigen load, positive alterations in the GI microbiota resulting in improved structure and function of the mucosal layer. EN is not generally recommended for UC patients during active disease; however, patients with poor oral intake or deficiencies may be candidates [68].

5 Emerging Nutrients Therapies and Bioactives

Specific nutrients and other dietary interventions may aid in managing acute IBD exacerbations and inducing and maintaining remission. This is an emerging area of research with probiotics, prebiotics, short-chain fatty acids (SCFAs), and omega-3 fatty acids being the most commonly investigated. In each instance, the mechanism of action is believed to be a reduction in the immune regulatory response.

a Probiotics

Probiotics are live microorganisms that when ingested in adequate amounts, confer health benefits on the host [73]. Probiotics are of interest to modify the microbiota population, modulate intestinal immunity and inflammation, and their ability to influence antigen tolerance, cytokine levels, and adhesion mechanisms [74–77]. UC and probiotics have been more thoroughly investigated compared to CD. VSL #3, a probiotic supplement containing *Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, and *Streptococcus thermophilus*, has shown efficacy in decreasing evidence and symptoms of active disease and inducing remission [78,79]. Similar effects, including decreased markers of inflammation, are reported with *L. delbrukeii* and *L. fermentum* in UC [80]. A recent meta-analysis concluded that probiotics are better at maintaining remission of UC compared to placebo [81]. Probiotics in the meta-analysis included VSL #3, *Bifidobacterium* species, *Lactobacillus* species, and *Escherichia coli* Nissle 1917. It is important to note that probiotic supplementation often occurs in tandem with traditional medicinal therapy; however, studies investigating *E. coli* Nissle 1917 suggest that it is an acceptable alternative to 5-aminosalicylic acid, an anti-inflammatory [82]. Unlike UC, results of the human intervention trials with probiotics for inducing or maintaining remission CD have not been as successful or as thorough as concluded by Cochrane Reviews [83–85]. Probiotics, including VSL #3, *L. rhamnosus*, and *Bifidobacterium*, also have considerable evidence for effectiveness in maintaining remission from pouchitis, the inflammatory state that occurs in the "pouch" surgically created from distal loops of ileum after colectomy [86–89].

b Prebiotics and SCFA

Prebiotics are functional polysaccharides that selectively stimulate growth of beneficial gut microbiota and are fermented to SCFA [90]. SCFA, specifically butyrate, have been shown to decrease markers of inflammation, primarily through decreasing NF-κB [91–94]. However, a recent study investigating the efficacy of fructooligosaccharides, a prebiotic, on active CD found no benefit compared to placebo [95]. Synbiotics, the combination of pre- and probiotics, have exhibited promising effects in both CD and UC [96–98].

c Omega-3 Fatty Acids

Fatty acids are unique in their ability not only to provide energy, but also to influence the inflammatory state in IBD and other inflammatory diseases. The Western diet greatly favors *n*-6 polyunsaturated fatty acids compared to *n*-3 fatty acids. Omega-6 and omega-3 fatty acids are incorporated into cell membranes and affect the physical, chemical, and functional properties of the cell and cell wall [99–101]. The lipids also serve as precursors for potent mediators in many physiologic reactions including the immune and inflammatory response. The *n*-6 and *n*-3 polyunsaturated fatty acids are metabolized to eicosanoids which are important in regulating the inflammatory state. Eicosanoids produced from *n*-6 fatty acids are proinflammatory and encourage blood clotting and thrombosis. Conversely, eicosanoids produced from *n*-3 fatty acids are antiinflammatory and decrease cellular adherence. Data from animal and human studies show that consumption of a high *n*-6:*n*-3 fatty acid ratio result in increased peripheral proinflammatory cytokines TNF-α, IL-6, and IL-1 concentrations, membrane permeability, edema, and increased reactive oxygen species, all consistent with an increased inflammatory state [102]. Similarly, diets containing *n*-3 fatty acids have been shown to decrease gene expression of inflammatory cytokines, increase anti-inflammatory resolvins, and alter signaling pathways in the inflammatory response, with the majority of the data in cardiovascular disease.

Clinical trial evidence for *n*-3 fatty acids in the management of IBD is conflicting. The recent Cochrane evidence-based reviews concluded that omega-3 fatty acids in both CD and UC did not improve maintenance of remission [103,104]. A trial by Nielsen and colleagues,

however, reports a decrease in proinflammatory cytokines after omega-3 supplementation compared to omega-6 during active CD [105]. Turner et al. recommend that future investigations utilize enteric coated capsules to insure intact delivery of the biologically active fatty acids to the inflamed tissue in the gut [104].

G Nutrition in IBD Remission

The majority of patients with IBD can achieve a full oral diet during disease remission. Individuals with extreme short bowel after surgical resection secondary to CD are the primary examples of who may require additional support to maintain nutritional status (see Section II on SBS). Characteristics of a healthy diet during IBD remission are similar to an individual without IBD. Fruits and vegetables are excellent sources of antioxidant nutrients and phytochemicals which may help to reduce oxidative stress. A low omega-6:omega-3 fatty acid ratio encourages production of antiinflammatory eicosanoids. Consuming sources of fermentable fiber will increase fermentation and therefore SCFA, including butyrate, production in the large intestine. Foods that are not well tolerated should be identified on an individual basis. Nutrition professionals can not only help identify food items that increase symptoms, but also offer suggestions on alternatives and label reading. Pre- and/or probiotics may be indicated for some patients. Small frequent meals can also help to increase tolerance. Individuals continuing to suffer from malabsorption or who are unable to consume and/or absorb adequate nutrients from food in their diet may require supplementation of macro- and micronutrients. CD patients in remission may benefit from EN support [106].

II SHORT BOWEL SYNDROME

A Definition

SBS is best defined functionally rather than by anatomical length of residual intestine. SBS is due to surgical resection of intestinal tissue resulting in a decrease in functional surface area and malabsorption [107–109]. Intestinal failure (IF) is defined as a decrease in functional intestinal mass below the amount required for digestion and absorption to meet the body's needs, be it growth in pediatric patients or maintenance in adult patients, and results in dependence on PN [110,111]. SBS is a common cause of IF wherein an individual's previously normal enteral nutrient intake is not enough to support growth and/or maintain weight due to malabsorption of macronutrients, micronutrients, fluid, electrolytes, and endogenous secretions. Deficiencies are common with the severity of such depending upon the amount and location of intestinal resection, age, and disease activity in the remaining tissue. The long list of complications attributed to SBS include weight loss, growth retardation, diarrhea, dehydration, electrolyte imbalances, loss of bone mass, renal oxalate stones, gallstones, lactic acidosis, and bacterial overgrowth, depending on the anatomical nature of the resection, function of the residual intestine, and age/growth status of the patient (see Fig. 39.4) [112,113].

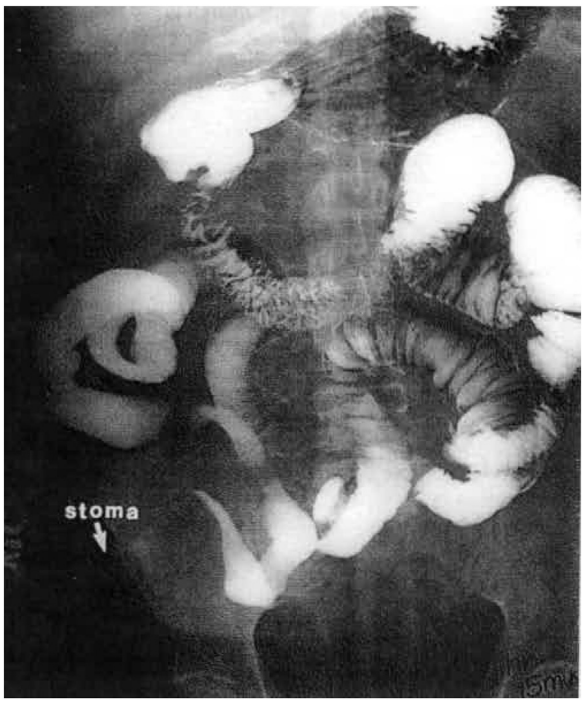

FIGURE 39.4 Radiologic image of patient following intestinal resection. *Reprinted from M. Sleisenger, M. Feldman, Sleisenger and Fordtran's Gastrointestinal and Liver Disease, seventh ed., W.B. Saunders, 2002, with permission from Elsevier.*

B Causes

In adults, the most common causes of SBS are mesenteric infarction, dysmotility, CD, obstruction, trauma, and radiation [114–117]. Causes of pediatric SBS can be prenatal, neonatal, or postnatal and include atresia, volvulus, Hirschsprung's disease, necrotizing enterocolitis, and IBD [110,111]. See Table 39.3 for causes of pediatric SBS.

C Predictors of the Severity and Prognosis in SBS

The prognosis for SBS can range from a temporary condition with less extreme intestinal resections or permanent in instances where little functional intestinal tissue remains. Symptoms appear immediately after resection and continue until sufficient adaptation occurs and/or

TABLE 39.3 Etiologies of Pediatric SBS

Prenatal	Neonatal	Postnatal
• Atresia (unique or multiple) • Apple peel syndrome • Midgut volvulus (malrotation) • Segmental volvulus (with Omphalomesenteric duct or intraabdominal bands) • Abdominal wall defects • Gastroschisis Ompjalocete • Extensive Hirschsprung's disease	• Midgut volvulus (midgut or segmental) • Necrotizing enterocolitis • Arterial thrombosis • Venous thrombosis	• Midgut volvulus (malrotation, bands, or tumor) • Complicated intussusception • Arterial thrombosis • IBD

Source: Reprinted from O. Goulet, F. Ruemmele, Causes and management of intestinal failure in children, Gastroenterology 130 (2 Suppl. 1) (2006) S116–S128, with permission from Elsevier.

medical, nutritional, or surgical interventions are successful. The length, location, and function of the remaining intestinal tissue and overall health of the patient greatly impact the need for nutrition support, the severity and duration of symptoms, and survival. Patients who survive without PN tend to have fewer adverse symptoms and are at decreased nutritional risk. It is well established that the ileum maintains a greater compensatory capacity when compared to the jejunum [118–120]. In addition, the ileocecal valve plays an integral role in releasing chyme from the small intestine to the large intestine and maintaining the balance of gut microbial populations in the colon [121]. Therefore, the patients with the following qualities have the best prognosis; patients who are very young have remaining distal ileum and ileocecal valve, have colon-in-continuity, and are otherwise healthy and well-nourished. Alternatively, qualities of a poor prognosis include loss of terminal ileum and ileocecal valve, advanced age at resection, loss of the colon in addition to small bowel, and/or presence of residual GI disease [112,122]. These patient attributes often result in dependence on large volumes of PN support, increased nutritional risk, and decreased quality of life.

D Intestinal Adaptation

Following intestinal resection, the remaining intestine undergoes dynamic structural and functional adaptation to compensate for the loss in tissue. Much of the data detailing intestinal adaptation is in animal models and includes lengthening and dilation of the residual bowel and increased proliferation [123–125]. This results in greater absorptive surface area through increased villus height and crypt depth (see Fig. 39.5). Importantly, nutrient transport is also unregulated. These adaptations are also observed in humans [119,126,127]. As mentioned previously, the ileum has the greatest potential for adaptation, although adaptation does occur through the length of the residual intestine [118,120].

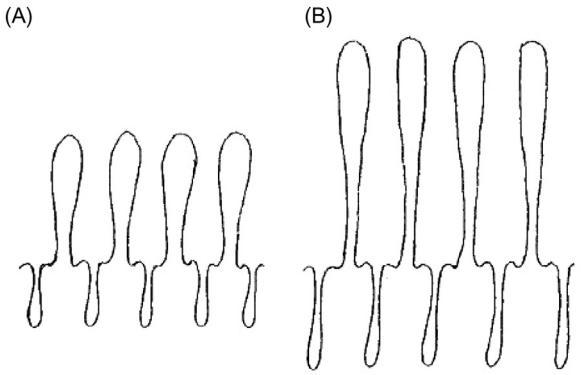

FIGURE 39.5 (A) Normal epithelium; (B) adapted epithelium with increased villus height and crypt depth following small bowel resection. *Reprinted from J.A. Vanderhoof, A.N. Langnas, Short-bowel syndrome in children and adults, Gastroenterology 113 (5) (1997) 1767–1778, with permission from Elsevier.*

E Massive Intestinal Resections in Infants

The same risk factors for increased morbidity apply in infants, including loss of ileum, ileocecal valve, and colon, except that infants adapt significantly better after resections than do adults. Healthy infants and children experience incredible intestinal growth from in utero until early adulthood. According to Weaver and colleagues, the small intestine doubles in length from 20 to 40 weeks gestation and nearly doubles again from term (275 cm) to age 10 (500 cm) [128]. Infants are reported to survive independent of PN with as little as approximately 30–60 cm of small bowel if the children have retained the ileocecal valve, and as little as 30–100 cm without the ileocecal valve [128,129]. When the ileocecal valve is lost, retained colon becomes more important for the

maintenance of electrolyte balance, adequate hydration, and to salvage malabsorbed substrates. For pediatric patients with extremely short lengths of residual functional intestine, who cannot fully adapt, several options exist. They can remain on PN, undergo small bowel lengthening procedures, or good candidates may receive a small bowel transplantation. PN, although lifesaving, imposes on the quality of life for the patient and caretakers and carries increased risk of infection, cholestasis and hepatic failure, gallstone formation, and nutrient deficiencies [130].

F Medical Management of SBS

Patients with SBS may be treated with several medications to improve symptoms, decrease GI secretions, delay gastric emptying, slow intestinal transit, and/or bind bile acids [131]. Antisecretory/antidiarrheal drugs such as proton pump inhibitors or histamine-2 receptor blockers are used in the initial stages of gastric acid hypersecretion that follows intestinal resection. Cholestyramine may aid in decreasing diarrhea by binding bile acids within the intestinal lumen and reduce the osmotic load present within the distal intestine [111]. Antimotility agents are utilized to slow transit time and extend the window of absorption. Conversely, prokinetic agents may be necessary to relieve symptoms such as abdominal distention and vomiting. Small intestine bacterial overgrowth is most commonly managed with antibiotic therapy. See Table 39.4 for a list of common medications utilized in SBS.

Glucagon-like peptide 2 (GLP-2) is an intestinotrophic hormone secreted from the distal intestine. A GLP-2 analog, teduglutide, was approved in 2012 by the United States Food and Drug Administration for use in SBS patients dependent on PN. GLP-2 or teduglutide has shown efficacy through increased villus height, crypt depth, urine output, wet weight absorption, with a decreased stomal energy output, PN volume, and overall increased body weight and lean body mass [132–137].

TABLE 39.4 Medical Management of SBS

Antimotility/Antidiarrheal
• loperamide, diphenoxylate-atropine
Prokinetic
• metoclopramide, erythromycin, cisapride
Immunosuppressive
• azathioprine, 6-mercaptopurine, methotrexate
Antisecretory
• proton-pump inhibitors, histamine-2 receptor blockers, clonidine, octreotide, cholestyramine
Antibiotics
• metronidazole
Trophic factors
• teduglutide

G Surgical Interventions in SBS

Although total PN is a lifesaving measure and has improved the prognosis for SBS and IF patients, serious complications exist including catheter sepsis and PN-associated liver disease. Surgical procedures will be considered when intestinal adaptation has been maximized and PN is still required. Currently, there are several surgical procedures performed to lengthen the residual intestine, which as a group are deemed autologous gastrointestinal reconstruction. In longitudinal intestinal lengthening and tailoring (LILT) or the Bianchi procedure, the dilated bowel is divided in half longitudinally, creating two loops of bowel, which are anastomosed together, doubling the intestinal length [138]. The serial transverse enteroplasty (STEP), first reported in a porcine model in 2003, is another lengthening procedure. STEP is similar to LILT with less handling of the mesentery and involves stapling the dilated bowel in a zigzag pattern to increase absorptive surface area [139,140]. Spiral intestinal lengthening and tailoring is a new procedure wherein the residual intestine is cut in a spiral pattern, stretched, and sutured to create a longer, narrower segment [141]. This procedure, while only performed in a few cases to date, has been successful in weaning or significantly decreasing PN requirements [142,143]. Small intestinal and multivisceral transplants have now become more successful and are indicated when patients experience PN failure with persistent complications including catheter-related infections and PN-associated liver disease [144]. There are several forms of small bowel transplant including small intestinal segments alone, liver-intestinal transplants, and multivisceral transplants [145].

H Nutrition Assessment in SBS

Nutrition assessment in the SBS patient is similar to that outlined for IBD, and indeed, many other clinical states. Determining the level of intestinal function in the residual tissue is a challenge in the clinical setting but assessing steatorrhea and fecal levels of macronutrients is possible [146]. Fecal or stoma output, serum electrolytes, and urine sodium content can also help establish hydration status [112,147]. Serum proteins such as albumin, transferrin, prealbumin, and hematological assessment can assist in establishing nutrition status and adequacy of macronutrient intake and absorption. Surgical history and radiologic examination can be utilized to determine the anatomical location and length of residual bowel, as this will significantly impact the patient's nutrition status. Careful consideration of the anatomical location of

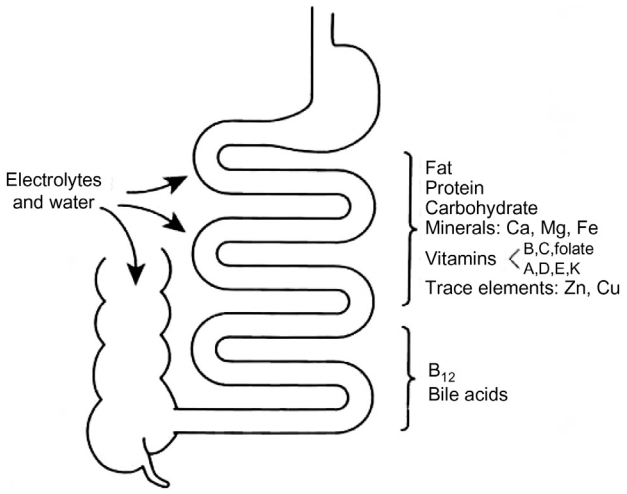

FIGURE 39.6 Sites of nutrient absorption in the healthy GI tract. *Reprinted from M. Sleisenger, M. Feldman, Sleisenger and Fordtran's Gastrointestinal and Liver Disease, seventh ed., W.B. Saunders, 2002, with permission from Elsevier.*

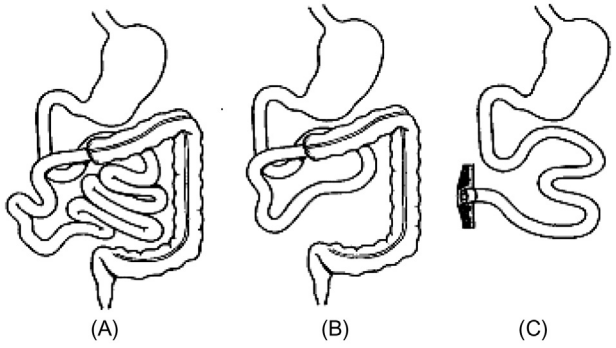

FIGURE 39.7 Common types of intestinal resection include (A) jejunal resections; (B) ileal resections; (C) end jejunostomy with resection of the ileum and colon. *Reprinted from M. Sleisenger, M. Feldman, Sleisenger and Fordtran's Gastrointestinal and Liver Disease, seventh ed., W.B. Saunders, 2002, with permission from Elsevier.*

resection and corresponding digestive and absorptive function is necessary for optimal nutrition therapy in SBS (see Fig. 39.6). Indeed, SBS is classified based on resected tissue, which aids in predicting the severity and nutritional risk and is discussed in the next section (see Fig. 39.7) [148].

I Medical Nutrition Therapy in SBS

The majority of SBS patients require PN following surgical resection, especially individuals with an end jejunostomy. The ultimate goal of nutrition support in the immediate postoperative phase is to restore and maintain hydration status. Depending on the stability of the patient and the amount of residual intestine, small amounts of oral nutrients may be provided [64]. During this time, urine volume, urine sodium content, and blood glucose should be monitored closely [149]. Enteral and/or oral nutrition should be initiated as soon as they are tolerated, with continued parenteral support as needed. Although there is no clearly superior formula in the literature, elemental enteral formulas may be beneficial in patients with rapid transit and severe malabsorption [68,150,151]. Electrolytes including sodium, potassium, and magnesium should be closely monitored.

Many patients, particularly those with an intact colon, progress to a fully oral diet. However, weaning from PN should not be considered until all other aspects of care are stabilized including medications and fluid management [152]. DiBaise and colleagues recommend patients achieve 80% of energy needs orally before complete PN weaning is initiated. Educational counseling with goal setting should occur before weaning is attempted. Individuals with colon-in-continuity are more likely to achieve independence from PN although patients without a colon may also be successful [153]. However, the colon does help in maintaining fluid balance and salvaging energy via fermentation by the gut microbiota. Depending on location of resection, micronutrients may need to be supplemented and should be closely monitored. Certain micronutrients such as magnesium and vitamin B_{12} may need to be administered intravenously if absorption is not adequate. Oral nutrition solutions or enteral feedings may be recommended if oral whole food intake is inadequate [68]. Adaptive hyperphagia, or oral consumption at least 1.5 times greater than calculated energy needs, occurs in most patients with SBS and may be helpful in the adaptive process and maintaining energy balance [154]. This biologic compensatory action should be encouraged, based on tolerance. The following sections discuss specific considerations for each type of SBS, depending on location of resection and remaining tissue.

1 Jejunoileal Anastomosis

The jejunum is the primary site of absorption for lipids, monosaccharides, peptides, and amino acids as well as a host of vitamins and minerals. As mentioned previously, the ileum has the capacity to adapt and restore much of the lost functionality over time [118,120]. With this in mind, jejunoileal anastomosis patients experience relatively mild malabsorption as long as the patient and the remaining GI tract are healthy. PN may be initially required but small frequent snacks and beverages may also be provided, as the presence of enteral nutrients encourages adaptation of the remaining intestine. Patients may experience a decreased lactose tolerance due to less lactase production from the resected jejunal tissue but restrictions should be recommended on an individual

basis depending on tolerance [148,155]. Ultimately, the patient should be able to consume oral meals with limited restrictions and minimal compromise in digestive and absorptive capacity.

2 Jejunocolic Anastomosis

Ileal resections present greater nutrition challenges compared to jejunal resections due to the decreased adaptive potential of the jejunum. Macronutrient and water malabsorption are more common. Although the ileum is not the major site of digestion and absorption of most nutrients, it is critical for vitamin B_{12}, remaining fat soluble vitamins, bile salts, and select acids. Because of these specialized ileal functions, B_{12} supplementation is often necessary [156] and the presence of steatorrhea and fat-soluble vitamin deficiencies must be monitored [156,157]. Since the colon remains in continuity, these patients may benefit from a higher complex carbohydrate diet through maximizing energy salvage by bacterial fermentation [158,159]. This increase in carbohydrates corresponds with a decrease in total energy from fat, which may be associated with decreased fluid and electrolyte fecal losses [159]. Fat intake from medium chain triglycerides may further improve absorption [160]. While adults consume approximately 2 L of fluid daily, the proximal small intestine secrete a much greater volume of 7–9 L per day [161]. While the healthy intestine is capable of reabsorbing the majority of this fluid, ileal resections result in increased risk of diarrhea and dehydration. Patients should be counseled to avoid high osmolarity fluids to decrease diarrheal exacerbations [162].

Patients with ileal resections are at increased risk of developing kidney stones. Under normal conditions, dietary oxalate binds calcium and other divalent cations, forming insoluble complexes. Following surgery, free oxalate becomes available for absorption in the colon. Kidney stones occur when the concentration of calcium and oxalate increase in the urine. Supplemental calcium and dietary oxalate restriction is often indicated to reduce absorption of oxalate and improve colonic fluid and electrolyte absorption [112,163]. Dietary sources of oxalate include berries, chocolate, coffee, tea, nuts, and green leafy vegetables such as kale and spinach.

3 Jejunostomy

Jejunostomy patients will face the greatest challenge to maintain energy and hydration status as loss of both the ileum and colon results in chronic malabsorption of macronutrients, micronutrients, and water, with relatively little possibility for adaptation. The same problems exist compared to jejunocolic SBS patients regarding nutrients that are absorbed specifically in the ileum including vitamin B_{12} and bile salts, but without the benefit of colonic energy salvage and fluid absorption. The fecal output contains large amounts of sodium and other electrolytes, resulting in increased risk of hypovolemia, hypokalemia, hypomagnesemia and other forms of nutrient depletion [112,115,163]. Jejunostomy patients should be encouraged to take in a greater amount of fluid than is lost from the ostomy and may benefit from oral rehydration solutions [163,164]. Ultimately these patients are likely to require long-term PN support [165].

J Nutrients for Intestinal Adaptation

There is great interest in strategically formulating the diet in SBS patients to maximize intestinal adaptation. Beginning soon after resection and continuing for months to several years, intestinal adaptation is a dynamic process that can be influenced by nutrition [130]. Much of the data for specific interventions is in animal models and includes adaptations such as increased bowel length and dilation, enhanced mucosal architecture including villus height and crypt depth yielding increased absorptive surface area, as well as increased nutrient transport [123–125]. There is also a body of evidence supporting these adaptations in humans [119,126,127].

1 Monomeric Versus Polymeric Enteral Formulas

Monomeric formulas consist of hydrolyzed amino acids while polymeric formulas contain whole proteins. An intermediate, semielemental formula is composed of oligopeptides averaging 4–5 amino acids in length. When considering monomeric and polymeric enteral formulas, the optimal enteral composition is controversial [110] and data are conflicting. Elemental formula has been reported to aid in weaning from PN in a small sample size of 4 pediatric SBS patients [166]; however a randomized, crossover double-blind trial of 10 pediatric SBS patients failed to detect a difference between a hydrolyzed and intact nutrient formula [167]. No clear choice between monomeric and polymeric formula emerges from the current literature, but polymeric formulas are generally more affordable and less likely to cause osmotic diarrhea [148].

2 Glutamine and Growth Hormone

Glutamine is an important energy source for enterocytes and has been investigated for inducing adaptation in SBS; however glutamine alone does not seem to be efficacious [168]. Glutamine is often supplemented in combination with growth hormone and benefits are reported in animal models such as increased remnant bowel length and improved architecture [169,170]. Human studies indicated an increase in plasma proteins, weaning from PN in a portion of studied patients and increased macronutrient and

electrolyte absorption [171–174]. Studies reporting no beneficial effect [175,176] and/or a return to baseline following treatment cessation also exist in the literature [172,177]. Adverse events attributable to growth hormone are also commonly reported [174–177]. In all, the evidence from an intestinal adaptation perspective is not promising and is not recommended [148]. In fact, a commercial growth hormone pharmaceutical targeted for SBS, Zorbtive, has seen limited success [178].

3 Fish Oil

Linolenic acid is an essential omega-3 fatty acid found in fish oil and is necessary to synthesize eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). EPA serves as a precursor to eicosanoids including thromboxanes, prostaglandins, and leukotrienes, which modulate inflammation and thrombosis, as discussed in the Section I. Pediatric IF-associated liver disease is a life-threatening complication associated with PN; however, promising evidence suggests that fish oil lipid emulsions may reduce the risk and even reverse the disease [179–182]. Fish oil, delivered both enterally or systemically, is also being investigated for its potential intestinotrophic properties. Studies utilizing intestinal resection rat models demonstrate an increase in structural and functional adaptation [183–185]. Fish oil as an intestinal adaptation modulator is a new concept, therefore well-designed human trials are lacking, with most studies being observational.

4 Butyrate

Acetate, propionate, and butyrate are the primary SCFAs produced in the human intestine through fermentation of otherwise nondigestible carbohydrates (including select prebiotic fibers) by anaerobic bacteria. Butyrate is a key energy source for colonocytes, and is absorbed with sodium and water and has intestinotrophic properties. Human studies are lacking, but strong animal data suggest that butyrate stimulates structural and functional adaptations of the residual intestine [186–189]. While the mechanism remains to be determined, it is hypothesized that GLP-2 may be involved.

5 Pre- and Probiotics

Pre- and probiotics are used alone and in combination in SBS, specifically for use in adaptation and managing small intestine bacterial overgrowth. Probiotic supplementation in rats including a mixture of *L. acidophilus*, *Bifidobacteria*, *S. thermophiles*, and *L. rhamnosus* GG (LGG) alone resulted in increased indices of intestinal architecture such as villus height and crypt depth [190,191]. Few intervention trials have been completed in humans with SBS; however, a double-blind, placebo-controlled, crossover clinical trial in pediatric SBS patients observed no effect of LGG on intestinal permeability [192]. A pediatric case study reports successful treatment of small intestine bacterial overgrowth with the synbiotic combination of *B. breve*, *L. casei*, and the prebiotic, galactooligosaccharides [193]. Interestingly, two other studies utilizing the same synbiotic treatment report decreased pathogenic bacteria in the feces, increased fecal SCFA, accelerated weight gain, and decreased nutrition support [194,195]. While these trials showed no negative impact of probiotic supplementation, there is concern about bacterial translocation, particularly with compromised barrier function in PN-dependent patients [196,197]. Overall, this evidence is not strong enough to support pre- and/or probiotic recommendations in SBS patients at this time [148].

III CONCLUSIONS

IBD and SBS are GI diseases with profound nutritional consequences. CD and UC are inflammatory conditions with an etiology yet to be determined while SBS is a functional disorder as the result of intestinal resection. Continued scientific advances in medicine and nutrition science provide a rich and ever-evolving body of knowledge that continues to improve the prognosis for individuals with IBD and SBS. In both IBD and SBS, diseases medical and surgical treatments are often necessary and can impact nutritional status. Medical nutrition therapy often takes the form of parenteral and/or EN but, in most cases, is temporary. Emerging research suggests that select nutrients can aid in decreasing inflammation in IBD and increase adaptation in SBS. It is imperative that clinicians carefully follow the nutrition intervention research in IBD and SBS to stay up-to-date on the most effective treatments.

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Chapter 40

Nutrient Considerations in Lactose Intolerance

Steve Hertzler[1], Dennis A. Savaiano[2], Abby Dilk[2], Karry A. Jackson[2], Sinead N. Bhriain[3] and Fabrizis L. Suarez[1]

[1]*Abbott Laboratories, Ross Products Division, Columbus, OH, United States,* [2]*Purdue University, West Lafayette, IN, United States,* [3]*Trinity College, University of Dublin, and Dublin Institute of Technology, Dublin, Ireland*

I INTRODUCTION

Ingestion of a large single dose of lactose (e.g., 50 g, the quantity in a quart of milk) by lactose maldigesters commonly results in diarrhea, bloating, and flatulence [1]. The wide dissemination of this information has led some of the lay population and a fraction of the medical community to attribute common gastrointestinal symptoms to lactose intolerance, independent of the dose of lactose ingested. As a result, a segment of the population avoids dairy products in the belief that even trivial doses of lactose will induce diarrhea or gas. However, multiple factors affect the ability of lactose to induce perceptible symptoms, including residual lactase activity [2], gastrointestinal transit time [3], lactose consumed with other foods [4], lactose load [5], and colonic fermentation [6]. The estimated 25% of adults in the United States who maldigest lactose are composed mainly of the Hispanic, Asian, and African-American populations (Table 40.1). These race/ethnic groups are rapidly growing segments of the population. Thus, the overall number of lactose maldigesters will grow in the United States in coming years. A major challenge for diet therapy of lactose maldigesters is to ensure adequate intakes of calcium, vitamin D, and other nutrients found largely in dairy products while at the same time minimizing the occurrence of lactose intolerance symptoms that would tend to limit milk consumption.

This chapter reviews the pathophysiology of lactose maldigestion, attempts to correct common misconceptions concerning the frequency and severity of lactose intolerance symptoms, and provides dietary strategies to minimize symptoms of intolerance.

II LACTOSE IN THE DIET

Lactose is the primary disaccharide in virtually all mammalian milks. It is unique among the major dietary sugars because of the β-1→4 linkage between its component monosaccharides, galactose and glucose. Lactose production in nature is limited to the mammalian breast, which contains the enzyme system (lactose synthase) necessary to create this linkage [7]. Human milk contains approximately 7% lactose by weight, which is among the highest lactose concentrations of all mammalian milks [5]. Cow's milk contains 4 or 5% lactose. Lactose, being water soluble, is associated with the whey portion of dairy foods. Thus, hard cheeses (with the whey removed from the curds) contain very little lactose compared with fluid milk. Table 40.2 shows the lactose content of selected foods.

In addition to food sources of lactose, small amounts of lactose are found in a wide variety of medications because of the excellent tablet-forming properties of lactose [5]. However, lactose is usually present in milligrams, rather than grams, quantities in most medications, and the amount is biologically insignificant for lactose maldigesters.

III DIGESTION OF LACTOSE

The small intestine is normally impermeable to lactose. Lactose must first be hydrolyzed to glucose and galactose, which are subsequently absorbed. Inability to digest lactose is referred to as *lactose maldigestion*. Lactose digestion is dependent on the enzyme lactase-phlorizin hydrolase (LPH), a microvillar protein that has at least three enzyme

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TABLE 40.1 Prevalence of LNP in Various Populations

Group	Prevalence (%)
Northern European	2–15
American White	6–22
Central European	9–23
Indian (Indian subcontinent)	
Northern	20–30
Southern	60–70
Hispanic	50–80
Ashkenazi Jew	60–80
Black	60–80
American Indian	80–100
Asian	95–100

Source: Used with permission from D.L. Swagerty, A.D. Walling, R.M. Klein, Lactose intolerance, Am. Fam. Physician 65 (2002) 1845–1850.

activities: β-galactosidase, phlorizin hydrolase, and glycosylceramidase [8,9]. Synthesis of LPH occurs in enterocytes, with the highest and most uniform synthesis being in the jejunum in humans [10]. The LPH gene is located on chromosome 2 and directs the synthesis of a pre-proLPH that is processed intracellularly (and possibly by pancreatic proteases) into the mature form that is anchored in the cell membrane at the brush border [11,12]. Lactase activity develops late in gestation compared to other disaccharidases. Lactase activity in a fetus at 34 weeks is only 30% that of a full-term infant, rising to 70% of the full-term activity by 35–38 weeks [13].

IV LOSS OF LACTASE ACTIVITY

Full-term infants possess high lactase activity, except for *congenital lactase deficiency*, in which lactase is completely absent at birth. Holzel et al. [14] first described congenital lactase deficiency in 1959. It is a very rare condition, such that even in Finland, where it is

TABLE 40.2 Lactose Content of Selected Foods

Product	Portion Size	Lactose Content (g/portion)
Milk, full fat	1 cup (244 g)	11
Milk, reduced fat (2%)	1 cup (244 g)	9–13
Milk, nonfat	1 cup (244 g)	12–14
Milk, chocolate	1 cup (244 g)	10–12
Buttermilk, fluid	1 cup (245 g)	9–11
Half and half	1 T. (15 g)	0.6
Yogurt, low fat	8 fl. oz. (227–258 g)	11–15
Cheese (blue, Camembert, cheddar, Colby, cream, Gouda, Limburger, grated Parmesan)	1 oz. (28 g)	0.1–0.8
Cheese, pasteurized processed (American, pimento, Swiss)	1 oz. (28 g)	0.4–1.7
Cottage cheese, whole	1 cup (210 g)	5–6
Cottage cheese, 2% fat	1 cup (226 g)	7–8
Butter	2 parts (10 g)	0.1
Ice cream, vanilla, regular	1 cup (133 g)	9
Ice milk, vanilla	1 cup (131 g)	10
Sherbet, orange	1 cup (193 g)	4

Source: Adapted with permission from J.D. Welsh, Diet therapy in adult lactose malabsorption: present practices, Am. J. Clin. Nutr. 31 (1978) 592–596.

most common, only 42 cases were diagnosed from 1966 to 1998 [11]. Lactase activity in jejunal biopsy specimens from infants with congenital lactase deficiency is reduced to 0–10 IU/g protein, and severe diarrhea results from unabsorbed lactose [11]. Treatment with a lactose-free formula eliminates symptoms and promotes normal growth and development [15].

Primary acquired hypolactasia, in which there is up to a 90–95% reduction in lactase activity, is much more common than congenital lactase deficiency (alactasia) [16]. The preferred term for this type of hypolactasia is *lactase nonpersistence* (LNP). It is estimated that approximately 75% of the world's population are LNP (see Table 40.1), with the exception of northern Europeans and a few pastoral tribes in Africa and the Middle East that maintain infantile levels of lactase throughout life [17]. Thus, LNP is not a "lactase deficiency" disease but is the normal pattern in human physiology, similar to the physiology of other mammalian species. This permanent loss of lactase occurs sometime after 3–5 years of age [9,18].

Lactase persistence is inherited as a highly penetrant, autosomal-dominant characteristic [17]. It has been hypothesized that individuals with a genetic mutation coding for lactase persistence would have gained a selective evolutionary advantage over LNP individuals in areas where dairy farming developed several thousand years ago [19,20]. Under marginal nutritional conditions, the individual with lactase persistence would be able to comfortably consume dairy products, deriving greater nutritional benefit. A key question has been whether the lactase persistence mutation in humans occurred before (the reverse cause hypothesis) or after (the cultural–historical hypothesis) the advent of dairying. DNA studies on the skeletal remains of predairy farming Neolithic Europeans [21] indicate that the most common allele for lactase persistence in Europeans (−13910*T) was not present, arguing for the cultural–historical hypothesis (this mutation is discussed in more detail in the following section). In addition, Tishkoff et al. [22] have identified three single-nucleotide polymorphisms (SNPs) for lactase persistence that developed in African populations as few as 3000 years ago. This date corresponds well with archeological data suggesting that cattle domestication came to different areas of Africa 3300–9000 years ago. These SNPs developed independently of the European mutation, providing striking evidence of both convergent evolution and the strong and relatively recent impact of a cultural practice such as dairy farming on the genome.

The genetic regulation of LPH has been studied extensively. Most evidence supports reduced levels of lactase mRNA in lactose maldigesters, suggesting that regulation is primarily at the level of transcription [23–26]. However, hypolactasia is sometimes present even when lactase mRNA is abundant, suggesting that posttranscriptional factors play a role [10,27,28]. One potential reason for conflicting results is the intestinal segment examined (duodenum vs jejunum). Lactase expression is higher and more uniform in the jejunum compared to the duodenum [29,30]. Another potential discrepancy is the age of the subjects studied. A poor correlation between lactase mRNA and lactase activity was reported in intestinal biopsies from children, although the biopsy specimens in this study were from duodenal sites [28]. Lactase activity in the jejunal enterocytes is found in a "mosaic"-type pattern [31]. In hypolactasic individuals, some jejunal enterocytes produce high amounts of lactase, whereas others, even those sharing the same villus, do not produce lactase [10]. Thus, rather than a uniform reduction in lactase production among all enterocytes, a hypolactasic individual may have a "patchy" distribution of lactose-producing enterocytes that are low in number relative to the nonlactase-producing enterocytes. In lactase-persistent individuals, all villus enterocytes may produce lactase. Current evidence suggests that the regulation of lactase is accomplished primarily at the level of transcription, although posttranscriptional factors (e.g., degradation of mRNA and posttranslational processing of the LPH protein) could be important in some individuals.

Secondary hypolactasia occurs as the result of damage to the enterocytes via disease, medications, surgery, or radiation to the gastrointestinal tract (Table 40.3) [5,32,33]. For example, the prevalence of microsporidiosis, which is associated with hypolactasia, can be as high as 50% in HIV-infected patients [34]. Seventy percent of HIV-infected patients showed evidence of lactose maldigestion compared to only 34% of controls [35]. In addition, the severity of lactose maldigestion increases in the more advanced stages of the disease. In general, secondary hypolactasia is reversible once the underlying cause is treated, but this reversal may require 6 months or more of diet therapy [5].

V DIAGNOSIS OF LACTOSE MALDIGESTION

A Genetic Testing

Historically, biochemical and/or symptom tests (described in Section V-B: Direct Assessment of Lactase Activity and Section V-C: Indirect Assessment Methods for Lactose Maldigestion) have been used to diagnosis lactose maldigestion. In approximately the past 5–10 years, however, the focus has been the development of genetic tests to identify markers of lactose maldigestion. These types

TABLE 40.3 Potential Causes of Secondary Hypolactasia

Disease		
Small Bowel	**Multisystem**	**Iatrogenic**
HIV enteropathy	Carcinoid syndrome	Chemotherapy
Regional enteritis (e.g., Crohn's disease)	Cystic fibrosis	Radiation enteritis
Sprue (celiac and tropical)	Diabetic gastropathy	Surgical resection of intestine
Whipple's disease (intestinal lipodystrophy)	Protein energy malnutrition	Medications
Ascaris lumbricoides infection	Zollinger–Ellison syndrome	Colchicine (antigout)
Blind loop syndrome	Alcoholism	Neomycin (antibiotic)
Giardiasis	Iron deficiency	Kanamycin (antibiotic)
Infectious diarrhea	Aminosalicylic acid (antibiotic)	
Short gut		

Sources: Adapted with permission from R. Srinivasan, A. Minocha, When to suspect lactose intolerance: symptomatic, ethnic, and laboratory issues, Postgrad. Med. 104 (3) (1998) 109–123; N.S. Scrimshaw, E.B. Murray, The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance, Am. J. Clin. Nutr. 48 (1998) 1083–1159; and D.A. Savaiano, M.D. Levitt, Milk intolerance and microbe-containing dairy foods, J. Dairy Sci. 70 (1987) 397–406.

of tests have several advantages over present methods. First, the measurements can be done on buccal cell samples, which are rapidly and easily obtained and require little or no preparation on the part of the individual being tested. Second, because the symptoms of lactose intolerance may often be confused with other gastrointestinal disorders, such as irritable bowel syndrome or Crohn's disease, genetic testing would allow for the ready differentiation of lactose maldigestion (resulting from LNP) from other gastrointestinal conditions. Enattah et al. [36] isolated two SNPs that are strongly associated with LNP in a primarily Finnish population. Both of these SNPs are located in a region adjacent to the lactase gene (LCT) on chromosome 2q21. The first, a substitution of cytosine for thymine 13,910 base pairs upstream of the 5′ end of LCT (termed C/T_{-13910}), was completely associated with biochemical evidence of LNP in 236 individuals. The second, g/A_{-22018}, was found in 229 of 236 cases. A subsequent study of intestinal biopsy samples showed that these SNPs coincided with low levels of mRNA for LPH consistent with transcriptional regulation [37]. It is likely that the C/T_{-13910} mutation impairs the binding of transcription factor Oct-1 [38]. The association between C/T_{-13910} and LNP was also observed in a study of children [39]. The presence of C/T_{-13910} had 100% specificity and 93% sensitivity in children older than 12 years compared with intestinal biopsy. Although these findings caused a great deal of excitement regarding the possibility of widespread genetic testing for LNP, other researchers have argued that genetic testing for LNP, based on C/T_{-13910}, is premature. In two reports of genetic testing of LNP among sub-Saharan African subjects, the C/T_{-13910} variant was extremely rare, and the authors suggested that other SNPs may be responsible for the LNP found in these populations [38,40]. The DNA regions encompassing the C/T_{-13910} LP/LNP variant have become the obvious targets in the search for additional lactose-persistent variants in such populations. T/G_{-13915} and G/C_{-14010} have been associated with lactose persistence in African populations [41,42], and Oct-1 has been shown to interact with T/G_{-13915} [43]. Novel substitutions are being continually found, and further studies are required to confirm the possible relationships of these substitutions to the lactose persistent trait [44,42]. The fact that individuals who are digesters have been found to carry no recognized causative allele is indicative of the fact that many more unidentified variants are in existence [45]. More research is required to determine the appropriate genetic markers of LNP in different populations before genetic testing becomes commonplace.

B Direct Assessment of Lactase Activity

Lactose digestion can be assessed directly or indirectly. The direct method involves obtaining a biopsy specimen of intestinal tissue and assaying for lactase activity or by intestinal perfusion studies [46]. Although these tests can accurately measure lactase activity, they are invasive and seldom used clinically.

C Indirect Assessment Methods for Lactose Maldigestion

The metabolic basis for different indirect tests of lactose maldigestion is shown in Fig. 40.1. Several indirect methods for assessing lactose digestion are available, including blood, urine, stool, and breath tests. Blood tests involve feeding a standard 50 g lactose dose and measurement of plasma glucose every 15−30 minutes over a period of 30 minutes to 2 hours. A rise in blood glucose of at least 25−30 mg/dL (1.5−1.7 mmol/L) is indicative of normal lactose digestion [41]. Unfortunately, blood glucose levels are subject to a variety of hormonal influences, reducing the reliability of this test. A blood test for galactose has been developed to correct this problem. The lactose dose is administered with a 500 mg/kg dose of ethanol (to prevent conversion of galactose to glucose in the liver) [46]. The galactose test is more reliable than the glucose test, but the ethanol exposure and somewhat invasive blood sampling are disadvantages.

A test has been devised to simultaneously measure intestinal lactose digestion (lactose digestion index [LDI]) and intestinal permeability (sugar absorption test [SAT]) [47]. The LDI/SAT consists of the oral administration of a 250-mL solution containing 25 g ^{13}C-lactose, 0.5 g ^{2}H-glucose, 5 g lactulose (a nonabsorbable disaccharide of galactose and fructose), and 1 g L-rhamnose. For the LDI test, the blood levels of ^{13}C-glucose and ^{2}H-glucose are measured before and at several time intervals after the LDI solution, and a low ^{13}C glucose:^{2}H-glucose ratio (<0.60) indicates significant failure to hydrolyze lactose. Urine collections more than 10 hours after the solution are obtained for measurements of the lactulose:rhamnose ratio, with a higher ratio indicating increased intestinal permeability. The LDI test was shown to outperform other measures of lactose digestion in one study [48] and it is less invasive than an intestinal biopsy, but it still has a number of limitations. The test requires the use of expensive isotopes and analytical equipment, involves both blood and urine collection, and has not been evaluated in persons with mucosal damage that might increase intestinal permeability.

A sometimes-used urine test involves the measurement of galactose in the urine, rather than the blood, during the lactose tolerance test with ethanol. Another urine test is conducted by simultaneously administering lactose and lactulose [46]. Small amounts of lactose (up to 1% of the ingested dose) and lactulose diffuse unmediated across the intestinal mucosa and are excreted in the urine. The ratio of lactose to lactulose in the urine (collected over 10 hours) is determined by the hydrolysis of lactose. A value of less than 0.3 indicates normal lactose digestion, and a ratio approaching 1.0 is observed in hypolactasia [46].

The measurement of stool pH and reducing substances in the stool has been used to assess lactose digestion in children. The analyses are easy to perform and convenient for the individuals being tested. However, stool pH has been shown to be unreliable in the diagnosis of hypolactasia in children and adults [46]. Furthermore, changes in gut motility and water excretion can alter the level of reducing substances in the stool. Thus, diagnosis of hypolactasia should not be based on stool tests alone [46].

Breath tests are the most widely used method for diagnosing carbohydrate maldigestion/malabsorption. The principle behind breath tests is that lactose, which escapes digestion in the small intestine, is fermented by bacteria in the colon, producing short-chain fatty acids (SCFAs) and hydrogen, carbon dioxide, and methane (in some

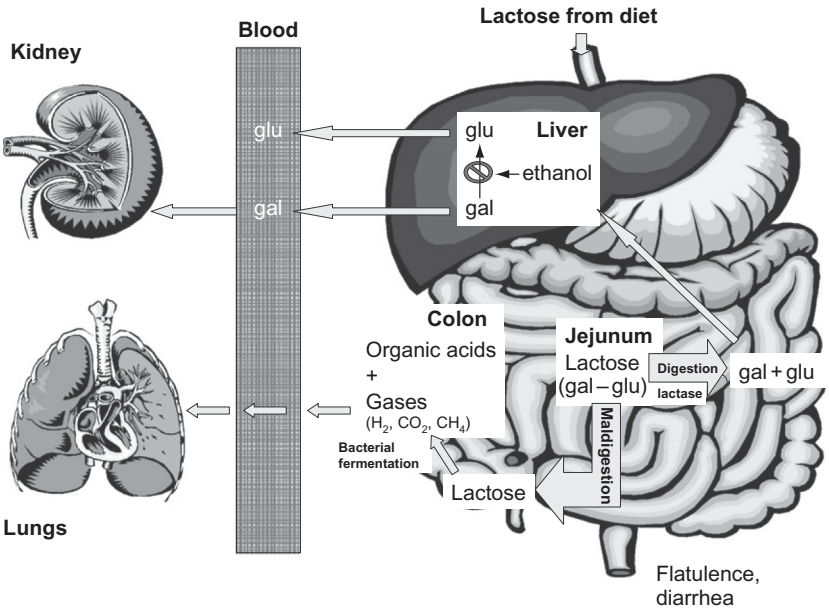

FIGURE 40.1 Metabolic background for understanding the diagnosis of lactose maldigestion and intolerance. gal, galactose; glu, glucose. *Adapted with permission from H. Arola, Diagnosis of hypolactasia and lactose malabsorption, Scand. J. Gastroenterol. 29 (Suppl. 202) (1994) 26−35.*

individuals). One breath test measures the amount of $^{13}CO_2$ excreted in the breath following administration of ^{13}C-lactose [46]. This stable isotope test has a safety advantage over older tests employing radioactive ^{14}C-lactose, but the high cost of the equipment prohibits widespread use of this method.

The current "gold standard" for diagnosis of carbohydrate maldigestion/malabsorption is the breath hydrogen test. Bacterial fermentation is the only source of hydrogen gas in the body. A portion of the hydrogen gas produced in the colon diffuses into the blood, with ultimate pulmonary excretion [49]. The hydrogen breath test is widely used because it is noninvasive and easy to perform. Typically, a subject is given an oral dose of lactose following an overnight (~12 hours) fast. Breath samples are collected at regular intervals for a period of 3–8 hours. In early studies, 50 g of lactose was used as a challenge dose. Almost all lactose maldigesters will experience intolerance symptoms following a dose of lactose this large [32], and yet many will be able to tolerate smaller, more physiologic doses of lactose. Doses of lactose that are in the range of 1 or 2 cups (240–480 mL) of milk (12–24 g lactose) have been more frequently used in recent years [50]. The dose of lactose used in the breath hydrogen test influences the diagnostic criterion for lactose maldigestion. Early studies with 50 g lactose showed perfect separation of lactose digesters from maldigesters using a rise in breath hydrogen of greater than 20 parts per million (ppm) above the fasting level [51]. Strocchi et al. [52] evaluated different criteria for diagnosis of carbohydrate maldigestion, using small doses of carbohydrate (10 g lactulose). Using a cut point of a 10-ppm or greater rise in breath hydrogen above fasting over an 8-hour period resulted in improved sensitivity (93% vs 76%) and only a slight decrease in specificity (95% vs 100%) compared to the 20-ppm cutoff. Furthermore, it was shown that using a sum of breath hydrogen values from hours 5, 6, and 7 and a 15-ppm or greater rise above fasting cut point resulted in 100% sensitivity and specificity.

Despite the advantages of breath hydrogen testing, care must be taken to ensure an accurate test. First, it is important to establish a low baseline breath hydrogen value, to which subsequent values are compared. This is accomplished by fasting before and after consumption of the lactose dose. In addition, it has been shown that a meal low in nondigestible carbohydrate (e.g., white rice and ground meat) the evening before the test results in lower baseline hydrogen [53]. Second, it is possible that some individuals may have a colonic microflora that is incapable of producing hydrogen. However, these individuals are rare, and the possibility of a nonhydrogen-producing flora can be ruled out by the administration of lactulose [52]. Third, approximately 40% of adults harbor significant numbers of methane-producing bacteria in the colon [49]. Because methanogenic bacteria consume four parts of hydrogen to produce one part methane [54], some authors have suggested that simultaneous measurement of methane will improve the accuracy of breath hydrogen testing in methane-producing subjects [55]. The availability of gas chromatographs that can analyze both hydrogen and methane in breath samples eliminates this potential problem. Finally, a number of factors (sleep, antibiotics, smoking, bacterial overgrowth of the small intestine, and exercise) may complicate the interpretation of breath hydrogen tests [46]. Therefore, standardization of the breath test protocol and appropriate controls are important.

Breath testing has been employed as a comparison to assess the clinical value of genetic testing. Positive breath tests have been correlated with both the C/T_{-13910} [56] and the g/A_{-22018} polymorphisms [57]. However, better correlation has been identified between the lactase-persistent genotype and a negative breath test [58]. Genetic testing does not provide information on actual patient symptoms, and the current usefulness of this test in populations with unestablished LP/LNP variants is questionable.

VI LACTOSE MALDIGESTION AND INTOLERANCE SYMPTOMS

A positive breath hydrogen test is indicative of lactose maldigestion. However, reduced lactase levels do not necessarily lead to intolerance symptoms. Symptoms of intolerance occur when the amount of lactose consumed exceeds the ability of both the small intestine and the colon to effectively metabolize the dose. Unhydrolyzed lactose passes from the small intestine to the large intestine, where it is fermented by enteric bacteria, producing the gases that are partially responsible for causing intolerance symptoms. The intensity of symptoms varies with the amount of lactose consumed [33,59–61], the degree of colonic adaptation [6,62], and the physical form of the lactose-containing food [63].

The correlation between lactose maldigestion and reported intolerance symptoms is unclear. Most maldigesters can tolerate the amount of lactose in up to 1 or 2 cups of milk without experiencing severe symptoms. However, some lactose maldigesters believe that small amounts of lactose, such as the amount used with coffee or cereal, cause gastrointestinal distress [64]. Individual differences observed in symptom reporting may reflect learned behaviors, cultural attitudes, or other social issues.

Lactose maldigesters, unselected for their degree of lactose intolerance, tolerated a cup of milk without experiencing appreciable symptoms [65–67]. However,

the results of these studies did not gain general acceptance, in part because of failure to enlist subjects with "severe" lactose intolerance. In 1995, Suarez et al. [64] conducted a study of 30 self-described "severely lactose intolerant individuals." Initial breath hydrogen test measurements indicated that approximately 30% (9 of 30) of the subjects claiming severe lactose intolerance were digesters and, thus, had no physiological basis for intolerance symptoms. These findings further demonstrate how strongly behavioral and psychological factors influence symptom reporting. Additional research is necessary to evaluate the psychological component of symptom reporting in lactose maldigesters.

VII LACTOSE DIGESTION, CALCIUM, AND OSTEOPOROSIS

Individuals who are lactose intolerant can generally tolerate moderate amounts of lactose with minimal or no gastrointestinal discomfort [64,68]. However, some lactose maldigesting individuals may unnecessarily restrict their intake of lactose-containing, calcium-rich dairy foods, thus compromising calcium intake. Milk and milk products contribute 73% of the calcium to the U.S. food supply [69]. Lactose maldigestion is associated with lower calcium intakes and is more frequent in osteoporotic cases than in controls [70–73]. For example, Newcomer et al. [70] found that 8 of 30 women with osteoporosis were lactose maldigesters compared to only 1 of 30 controls. In addition, calcium intakes of postmenopausal women positive for LNP in this study were significantly lower than in the lactase-persistent women (530 vs 811 mg/day). Interestingly, in this report, and another by Horowitz et al. [71], few of the LNP subjects reported a history of milk intolerance and yet they still restricted milk intake. The lower milk intakes in these subjects may have been due to factors other than lactose intolerance. However, it is also possible that these subjects restricted their milk intakes because of lactose intolerance in childhood, forgot that they had done so, and simply maintained that pattern of milk intake throughout life.

Another potential explanation for the increased prevalence of osteoporosis among lactose maldigesters is that maldigestion of lactose might decrease the absorption of calcium. Human and animal studies suggest that lactose stimulates the intestinal absorption of calcium [69]. However, there is considerable disagreement regarding the influence of lactose and lactose maldigestion on calcium absorption in adults. This disagreement results from a number of factors, including the dose of lactose given, the choice of method for assessing calcium absorption (single isotope, double isotope, and balance methods), prior calcium intake of the subjects, and the form in which the calcium is given (milk vs water).

Kocian et al. [74], using a single-isotope (^{47}Ca) method, demonstrated improved absorption of a 972-mg calcium dose from lactose-hydrolyzed milk compared to milk containing lactose in lactose maldigesters. Conversely, the regular milk resulted in increased calcium absorption versus the lactose-hydrolyzed milk in lactose digesters. Another study—using dual-isotope methods, a 50 g lactose load, and 500 mg of calcium chloride in water—found similar results [75]. Total fractional calcium absorption was decreased in maldigesters and increased in digesters with lactose feeding. However, the doses of lactose given in these studies (39–50 g or the equivalent of 3 or 4 cups of milk) were unphysiologic and may have resulted in more rapid intestinal transit than would be observed with more physiologic amounts of lactose.

Several studies have been conducted with physiologic doses of lactose. Griessen et al. [76], using dual-isotope methods, found that lactose maldigesters ($n = 7$) had a slightly, but not statistically significant, greater total fractional calcium absorption from 500 mL of milk compared to 500 mL of lactose-free milk. They also observed a nonsignificant decline in fractional calcium absorption in normal subjects ($n = 8$) when comparing lactose-free milk with regular milk. In another dual-isotope study, lactose maldigesters absorbed more calcium from a 240 mL dose of milk than did digesters ($\sim$35% vs 25%), which was thought to be due to lower calcium intakes in the lactose maldigesting group [77]. Most important, however, no difference was observed in fractional calcium absorption between lactose-hydrolyzed and regular milk in either group of subjects. Calcium absorption from milk and yogurt, each containing 270 mg of calcium, was studied using a single-isotope method [78]. No significant differences were observed in calcium absorption between milk and yogurt in either the lactose maldigesting or digesting subjects. Interestingly, yogurt resulted in slightly, but significantly ($p < 0.05$), greater calcium absorption in lactose maldigesters compared to lactose digesters.

Differences in study methodology (milk vs water, dose of lactose, and the choice of method for determining calcium absorption) may explain contrasting results. Physiologic doses of lactose (e.g., amounts provided by up to 2 cups of milk) are not likely to have a significant impact on calcium absorption. The increased prevalence of osteoporosis in lactose maldigesters is most likely related to inadequate calcium intake rather than impaired intestinal calcium absorption.

VIII DIETARY MANAGEMENT FOR LACTOSE MALDIGESTION

It is difficult for lactose maldigesters to consume adequate amounts of calcium if dairy products are eliminated from the diet. Fortunately, lactose intolerance is easily

TABLE 40.4 Dietary Strategies for Lactose Intolerance

Factors Affecting Lactose Digestion	Dietary Strategy	Reference
Dose of lactose	Consume a cup of milk or less at a time, containing up to 12 g lactose.	Suarez et al. [64]
		Hertzler et al. [6]
		Suarez et al. [68]
Intestinal transit	Consume milk with other foods, rather than alone, to slow the intestinal transit of lactose.	Solomons et al. [85]
		Martini and Savaiano [4]
		Dehkordi et al. [86]
Yogurt	Consume yogurt containing active bacteria cultures. One serving, or even two, should be well tolerated. Lactose in yogurts is better digested than the lactose in milk. Pasteurized yogurt does not improve lactose digestion; however, these products, when consumed, produce few to no symptoms.	Kolars et al. [93]
		Gilliland and Kim [100]
		Savaiano et al. [63]
		Shermak et al. [99]
		Savaiano et al. [63]
		Kolars et al. [93]
		Gilliland and Kim [100]
Digestive aids	Over-the-counter lactase supplements (pills, capsules, and drops) may be used when large doses of lactose (>12 g) are consumed at once. Lactose-hydrolyzed milk is also well tolerated.	Moskovitz et al. [119]
		Lin et al. [114]
		Ramirez et al. [120]
		Nielsen et al. [126]
		Biller et al. [131]
		Rosado et al. [128]
		Brand and Holt [124]
Colon adaptation	Consume lactose-containing foods daily to increase the ability of the colonic bacteria to metabolize undigested lactose.	Perman et al. [138]
		Florent et al. [62]
		Hertzler et al. [6]

managed. Dietary management approaches that effectively reduce or eliminate intolerance symptoms are discussed next and shown in Table 40.4.

A Dose–Response to Lactose

There is a clear relationship between the dose of lactose consumed and the symptomatic response. Small doses (up to 12 g of lactose) yield no symptoms [1,64,66–68], whereas high doses (>20–50 g of lactose) produce appreciable symptoms in most individuals [1,79–81]. In a well-controlled trial, Newcomer et al. [1] demonstrated that more than 85% of lactose maldigesters developed intolerance symptoms after consuming 50 g of lactose (the approximate amount of lactose in 1 quart of milk) as a single dose. The frequency of reported symptoms may be attributed to the nonphysiologic nature of the lactose dose and the physical form of lactose load administered. A lactose dose of 15–25 g produces appreciable symptoms in some subjects [30,74]. The incidence of symptom reporting generally remains higher than 50% with intermediate doses. However, the frequency varies from less than 40% to greater than 90% [32]. In a double-blind protocol, Suarez et al. [64] demonstrated that feeding 12 g of lactose with a meal resulted in minimal to no symptoms in maldigesters. Interestingly, in unblinded studies [82,83], lactose maldigesters more frequently reported intolerance symptoms after consuming lactose loads similar to those given by Suarez et al. Subsequently, Suarez et al. [68] provided further evidence that individuals who are lactose intolerant can consume lactose-containing foods without experiencing appreciable symptoms by

feeding lactose maldigesters 2 cups of milk daily. One cup of milk was given with breakfast, and the second was given with the evening meal. The symptoms reported by maldigesters after consumption of 2 cups of milk a day were trivial.

Symptoms from excessive lactose in the intestine may increase out of proportion to dose, which raises the possibility that the absorption efficiency decreases with increased loads. Fractional lactose absorption is most likely influenced by dose, with more effective absorption of small loads and less effective utilization of larger doses. Hertzler et al. [60], using breath hydrogen as an indicator, suggested that 2 g of lactose is almost completely absorbed, whereas there was some degree of maldigestion when a 6 g load was ingested. The only study directly measuring the lactose absorption efficiency in lactose maldigesting subjects is that of Bond and Levitt [84], who intubated the terminal ileum and then fed the subjects ^{14}C-lactose mixed with polyethylene glycol, a nonabsorbable volumetric marker. Analysis of the ratio of ^{14}C-lactose to polyethylene glycol passing through the terminal ileum allowed researchers to calculate the percentage of lactose absorbed. On average, maldigesters absorbed approximately 40% of a 12.5 g lactose load, whereas the other 60% passed to the terminal ileum. However, sizable differences were seen in absorption efficiency among lactose maldigesters. These differences may represent differences in residual lactase efficiency and/or gastric emptying and intestinal transit time.

B Factors Affecting Gastrointestinal Transit of Lactose

Consuming milk with other foods can minimize symptoms from lactose maldigestion [4,85,86]. A probable explanation for these findings is that the presence of additional foods slows the intestinal transit of lactose. Slowed transit allows more contact between ingested lactose and residual lactase in the small intestine, thus improving lactose digestion. It is also possible that additional foods may simply slow the rate at which lactose arrives in the colon because a delay in peak breath hydrogen production, rather than a significant decrease in total hydrogen production, has been reported [4]. The slower fermentation of lactose might allow for more efficient disposal of fermentation gases, reducing the potential for symptoms.

The energy content, fat content, and added components such as chocolate may influence gastrointestinal transit of lactose and subsequent lactose digestion. Leichter [87] showed that 50 g of lactose from whole milk (1050 mL) resulted in fewer symptoms (abdominal discomfort, bloating, and flatulence) compared to 50 g of lactose from either skim milk (1050 mL) or an aqueous solution (330 mL). However, only blood glucose was measured to determine lactose digestion, and no statistical evaluation of symptoms was done in this study. Other studies have demonstrated that higher fat milk may slightly decrease breath hydrogen relative to skim milk [86] but not improve tolerance [86,88,89]. Furthermore, increasing the energy content or viscosity of milk has not been effective in improving lactose digestion or tolerance [90,91].

Chocolate milk has been recommended for individuals who are lactose intolerant. Apparently, chocolate milk empties from the stomach more slowly than unflavored milk, possibly because of its higher osmolality or energy content [3]. Two reports [86,92] have demonstrated improved lactose digestion (i.e., reduced breath hydrogen) following consumption of chocolate milk. One of these studies [92] documented fewer symptoms in subjects.

Clearly, consumption of milk with other foods results in improved tolerance compared to consumption of milk alone. Therefore, consuming small amounts of milk routinely with meals is a recommended approach for individuals who are lactose intolerant to obtain sufficient calcium from dairy products. These individuals may also try chocolate milk to improve tolerance.

C Yogurt

The lactose in yogurt with live cultures is digested better than lactose in milk and is well tolerated by those who are lactose intolerant [93]. In fact, Kolars et al. [93] reported only 20% of participants who had consumed yogurt experienced diarrhea or flatulence, as compared to 80% of participants given a similar quantity of lactose in milk. Prior to fermentation, most commercially produced yogurt is nearly 6% lactose because of the addition of milk solids to milk during yogurt production. However, as the lactic acid bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) multiply to nearly 100 million organisms per milliliter, 20–30% of the lactose is utilized, decreasing the lactose content of yogurt to approximately 4% [94]. During fermentation, the activity of the β-galactosidase (lactase) enzyme substantially increases. Casein, calcium phosphate, and lactate in yogurt act as buffers in the acidic environment of the stomach, thus protecting a portion of the microbial lactase from degradation and allowing the delivery of intact cells to the small intestine [95,96]. As yogurt enters the small intestine, the increasing pH accompanied by a slower transit time maintains the activity of bacterial lactase, thereby allowing for adequate lactose digestion and the prevention of lactose maldigestion symptoms [97]. In the duodenum, once the intact bacterial cells interact with bile acids, they are disrupted, allowing substrate access to enzyme activity.

Yogurt consumption results in enhanced digestion of lactose and improved tolerance [63,93,98–100]. In 1984,

Kolars et al. [93] and Gilliland and Kim [100] reported enhanced lactose digestion from yogurt in lactose maldigesters. In both studies, breath hydrogen excretion was significantly reduced with the consumption of live culture yogurt. Furthermore, Kolars et al. found that an 18-g load of lactose in yogurt resulted in significantly fewer intolerance symptoms reported by subjects compared to the other forms of lactose given. Also in 1984, Savaiano et al. [63] demonstrated that yogurt feeding resulted in one-third to one-fifth less hydrogen excretion compared to other lactose-containing dairy foods with no symptoms. Shermak et al. [99] reported that a 12 g load of lactose in yogurt resulted in lower peak hydrogen in children with a delay in the time for breath hydrogen to rise compared to a similar lactose load given in milk. These children experienced significantly fewer intolerance symptoms with yogurt consumption.

Yogurt pasteurization following fermentation has been somewhat controversial [100]. One advantage of pasteurizing yogurt is a longer shelf life. However, removing the active cultures that are partly responsible for improved lactose digestion may increase intolerance symptoms and cause lactose maldigesters to avoid yogurt products. Further, galactose absorption and intestinal lactase activity were enhanced in rats fed live culture yogurt as compared to rats fed pasteurized yogurt [101]. Pasteurizing yogurt increases the maldigestion of lactose [63,99,100]. However, pasteurized yogurt is moderately well tolerated, producing minimal symptoms [63,98,99]. Because pasteurized yogurt is relatively well tolerated, other factors such as the physical form, or gelling, and the energy density of yogurt may play a role in tolerance. The level of the β-galactosidase enzyme in yogurt may not be the limiting factor for improving lactose digestion because not all yogurts have the same level of lactase activity [102]. Martini et al. [102] fed yogurts with varying levels of microbial β-galactosidase. The remaining characteristics of the test yogurts (pH, cell counts, and lactose concentrations) were similar. Despite the different levels of β-galactosidase activity, all yogurts improved lactose digestion and minimized intolerance symptoms.

Whether yogurt is flavored or unflavored is an impactful characteristic in regards to lactose digestion [103]; via breath hydrogen measurements, unflavored yogurt resulted in hydrogen production of 37 ppm/hour in lactase-deficient individuals, whereas flavored yogurt produced an intermediate hydrogen reading of 77 ppm/hour. This difference may be attributed to an increased osmotic effect of sugar in the stomach, dilution of the yogurt via flavoring or sugar, or inhibition of glucose as the end product of digestion. Despite the increased breath hydrogen readings for lactase-deficient individuals who consumed flavored yogurt, they had no symptoms.

D Kefir

Kefir is a fermented dairy beverage that originated in Eastern Europe and has been made for centuries. Unlike yogurt, it is drinkable, and the kefir grains used to culture the milk contain a wider variety of starter culture microorganisms. Hertzler and Clancy [104] investigated the effect of kefir ingestion on lactose maldigestion and intolerance symptoms in a group of lactose maldigesters. The kefir used in the study contained *S. lactis*, *L. plantarum*, *S. cremoris*, *L. casei*, *S. diacetylactis*, *Saccharomyces florentinus*, and *Leuconostoc cremoris* (as per label information) as the starter cultures. Feeding a 20 g lactose load as plain kefir reduced breath hydrogen excretion (8-hour area under the curve) by nearly threefold and decreased flatulence symptoms by 50% compared with the same amount of lactose from milk. The response to yogurt was similar to that observed with kefir.

E Unfermented Acidophilus Milk

Individuals who are lactose maldigesters may consume unfermented milk containing cultures of *L. acidophilus* in an effort to consume adequate amounts of calcium and avoid intolerance symptoms [105–107]. Various strains of *L. acidophilus* exist; however, strain NCFM has been most extensively studied and used in commercial products. Unfermented acidophilus milk tastes identical to unaltered milk because the NCFM strain does not multiply in the product, provided that the storage temperature is below 40°F (5°C) [105,106,108]. *Lactobacillus acidophilus* strain NCFM is derived from human fecal samples [95] and contains β-galactosidase. The effectiveness of acidophilus milk on improving lactose digestion and intolerance symptoms has been evaluated. Most evidence suggests that unfermented acidophilus milk does not enhance lactose digestion or reduce intolerance symptoms [63,109–111], primarily because of the low concentration of the species in the milk. Improved lactose digestion has been observed by some [111]; however, the test milk in this study contained a much higher concentration of *L. acidophilus* than is normally used to produce commercial acidophilus milks.

Furthermore, the microbial lactase from *L. acidophilus* may not be available to hydrolyze the lactose in vivo [103,106,110]. *Lactobacillus acidophilus* is not a bile-sensitive organism [95,105]. Therefore, once the intact bacterial cells reach the small intestine, bile acids may not disrupt the cell membrane to allow the release of the microbial lactase. However, sonicated acidophilus milk improved lactose digestion by reducing breath hydrogen [112]. Thus, if less bile-resistant strains were developed and used in adequate amounts, these strains may allow the β-galactosidase to be released, possibly yielding an

effective approach to the dietary management of lactose maldigestion.

Finally, fermented and unfermented milks containing bifidobacteria may also be useful in the management of lactose intolerance. Studies of milks treated with *Bifidobacterium bifidus* GD428 [103] and *B. longum* (strains B6 and 15708) [113] revealed lower breath hydrogen excretion and flatulence symptoms compared with milk in lactose maldigesters. However, the beneficial effects, as with acidophilus milk, were still less than those observed with yogurt.

F Lactase Supplements and Lactose-Reduced Milk

Lactase pills, capsules, and drops contain lactase derived from yeast (*Kluyveromyces lactis*) or fungal (*Aspergillus niger* and *A. oryzae*) sources. Common brands include Lactaid and Lacteeze. Dosages of lactase per pill or caplet vary from 4000 to 9000 FCC (Food Chemical Codex) units [114–116]. Since 1984, these over-the-counter preparations have been given generally recognized as safe status by the U.S. Food and Drug Administration [117]. Lactase pretreated milks (100% lactose hydrolysis) are available from Lactaid and Dairy Ease [115,118].

A number of studies have evaluated the effectiveness of these products. Doses of 3000–6000 FCC units of lactase administered just prior to milk consumption decrease both breath hydrogen and symptom responses to lactose loads ranging from 17 to 20 g [114,119,120]. The decrease in breath hydrogen and symptoms is generally dose dependent. Doses up to 9900 FCC units may be needed for digestion of a large lactose load, such as 50 g of lactose [114,121,122].

Lactose-hydrolyzed milks also improve lactose intolerance symptoms in both children and adults [80,81,123–133]. A by-product of lactose-hydrolyzed milk is increased sweetness due to the presence of free glucose [64]. This increased sweetness may increase its acceptability in children [126].

G Colonic Fermentation and Colonic Bacterial Adaptation of Lactose

The colonic bacteria ferment undigested lactose and produce SCFA and gases. Historically, this fermentation process was viewed negatively as a cause of lactose intolerance symptoms. However, it is now recognized that the fermentation of lactose, as well as other nonabsorbed carbohydrates, plays an important role in the health of the colon and affects the nutritional status of the individual.

The loss of intestinal lactase activity in lactose maldigesters is permanent. Studies from Israel, India, and Thailand have reported that feeding 50 g of lactose or more per day for periods of 1–14 months has no impact on jejunal lactase activity [17,134,135]. Despite this observation, milk has been used successfully in the treatment of malnourished children in areas of the world where lactose maldigestion is common. In Ethiopia, for example, 100 schoolchildren, aged 6–10 years, were fed 250 mL of milk per day for a period of 4 weeks [136]. Although the children initially experienced some degree of gastrointestinal symptoms, the symptoms rapidly abated and returned to pretrial levels within 4 weeks. Similar results were observed with schoolchildren in India [134]. Finally, a study of African-American males and females aged 13–39 years who were lactose maldigesters and lactose intolerant showed that 77% of the subjects could ultimately tolerate 12 g or more of lactose if lactose was increased gradually and fed daily over a period of 6–12 weeks [137]. Approximately 80% of the subjects (18 of 22) had rise in breath hydrogen of at least 10 ppm above baseline at the maximum dose of lactose tolerated, suggesting that improved digestion of lactose in the small intestine was not responsible for the increased tolerance. Therefore, the authors proposed that colonic bacterial adaptation was a likely explanation for these findings.

Evidence for colonic bacterial adaptation to disaccharides (lactulose and lactose) is substantial. Perman et al. [138] fed adults 0.3 g/kg lactulose per day for 7 days and observed a decrease in fecal pH from 7.1 ± 0.3 to 5.8 ± 0.6. The breath hydrogen response to a challenge dose of lactose (0.3 g/kg) fell significantly after lactulose adaptation. Employing the same experimental design and doses of lactulose, Florent et al. [62] measured fecal β-galactosidase, colonic pH, breath hydrogen, fecal carbohydrates, SCFA, and ^{14}C-lactulose catabolism in subjects before and after the 7-day lactulose maintenance period. Fecal β-galactosidase was six times greater after lactulose feeding, and breath hydrogen fell significantly. Breath $^{14}CO_2$ (indicating catabolism of ^{14}C-lactulose) increased and fecal outputs of lactulose and total hexose units were low after the lactulose feeding. Symptoms were not measured; however, a follow-up study showed that adaptation to lactulose (40 g/day for 8 days) reduced symptoms of diarrhea induced by a large dose (60 g) of lactulose [139]. Breath hydrogen decreased significantly, and fecal β-galactosidase activity increased as in the previous study.

Several feeding trials directed to adapting lactose maldigesters to lactose have been reported. The first was a blinded, crossover study conducted at the University of Minnesota [6]. Feeding increasing doses of lactose (from 0.3 to 1.0 g/kg/day) for 16 days resulted in a threefold increase in fecal β-galactosidase activity, which returned to baseline levels within 48 hours after substitution of dextrose for lactose. Furthermore, 10 days of lactose feeding (from 0.6 to 1.0 g/kg/day), compared to dextrose

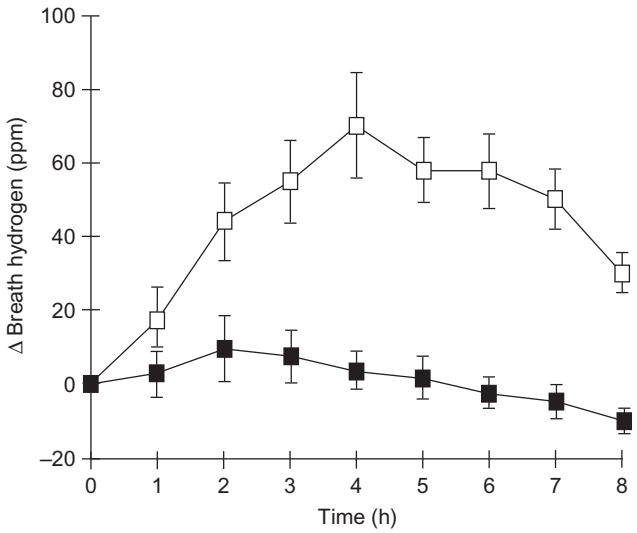

FIGURE 40.2 Breath hydrogen response to a lactose challenge after lactose (□) or dextrose (■) feeding periods. Data are the means ±SEM, $n = 20$. *Reprinted with permission from S.R. Hertzler, D.A. Savaiano, Colonic adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance, Am. J. Clin. Nutr. 64 (1996) 232–236.*

feeding, dramatically decreased the breath hydrogen response to a lactose challenge dose (0.35 g/kg; Fig. 40.2). After lactose adaptation, the subjects no longer appeared to be lactose maldigesters, based on a 20-ppm rise in breath hydrogen above fasting. The large doses of lactose fed during the adaptation period (averaging 42–70 g/day) resulted in only minor symptoms. In addition, the severity and frequency of flatus symptoms in response to the lactose challenge dose were reduced by 50%.

The second study was a double-blind, placebo-controlled trial conducted in France with a group of 46 subjects who were lactose intolerant [140]. Following a baseline lactose challenge with 50 g of lactose, subjects were randomly assigned to either a lactose-fed group ($n = 24$) or a sucrose-fed control group ($n = 22$). Subjects were fed 34 g of either lactose or sucrose per day for 15 days. Fecal β-galactosidase increased and breath hydrogen decreased as a result of lactose feeding. Clinical symptoms (except diarrhea) were 50% less severe after lactose feeding. However, the sucrose-fed control group also experienced a comparable decrease in symptoms, despite no evidence of metabolic adaptation. Thus, these authors concluded that the improvements in symptoms resulted from familiarization with the test protocol rather than from metabolic adaptation.

Lactose digesters and maldigesters who reported milk aversion could improve their status of milk consumption as the result of a 21-day intervention [141]. The intervention called for having participants consume 1/2 cup of milk twice a day during the first week, 2/3 cup of milk twice a day during the second week, and 1 cup of milk during the third week. All participants had a decrease in symptoms associated with lactose maldigestion including abdominal pain, bloating, diarrhea, flatulence, and headache. Most notably, however, maldigesters experienced a decrease in symptoms to a greater degree than digesters. Three months following the intervention, participants increased milk consumption by approximately 10 servings per month, which improved their calcium intake category from poor (<700 mg/day) to far (700–1000 mg/day). Milk consumption decreased slightly to 6 servings per month at the 6-month follow-up; however, the behaviors and feelings participants' had toward milk became more consistent. Thus, a similar intervention could be implemented for individuals who maintain their avoidance of dairy products after experiences with lactose intolerance during childhood.

Colonic bacteria develop an increased ability to ferment lactose (indicated by increased fecal β-galactosidase) following prolonged lactose feeding. Because hydrogen gas is an end product of fermentation, it might be expected that the increased ability to ferment lactose would result in an increase, rather than the observed decrease, in breath hydrogen. However, breath hydrogen excretion represents the net of bacterial hydrogen production and consumption in the colon [54]. A decrease in net production of hydrogen could result from either decreased bacterial production or increased consumption. To examine the mechanism for decreased breath hydrogen after lactose adaptation, Hertzler et al. [142] employed metabolic inhibitors of bacterial hydrogen consumption (methanogenesis, sulfate reduction, and acetogenesis) to obtain measures of absolute hydrogen production. Subjects were fed increasing amounts of lactose or dextrose in a manner similar to previous studies. Fecal samples were assayed in vitro for absolute hydrogen production and hydrogen consumption. Absolute hydrogen production after 3 hours of incubation with lactose was threefold lower after lactose adaptation (242 ± 54 mL) compared to the dextrose feeding period (680 ± 7 mL, $p = 0.006$). Fecal hydrogen consumption was unaffected by either feeding period. These findings tend to support the hypothesis that prolonged lactose feeding favors the growth or metabolic activity of bacteria (e.g., bifidobacteria and lactic acid bacteria) that can ferment lactose without the production of hydrogen. Feeding lactose, lactulose, and nonabsorbable oligosaccharides stimulates the proliferation of lactic acid bacteria in the colon [143–145]. In addition, high populations of bifidobacteria inhibit the growth of known hydrogen-producing organisms, such as Clostridia or *Escherichia coli* [146].

Colonic bacterial adaptation to lactose does occur. Although the role of colonic adaptation in improving symptoms is not firmly established, it is clear that many individuals who are lactose intolerant can develop a tolerance to milk if they consume it regularly. This may represent a simpler and less expensive solution than the use of lactose digestive aids. A product called Lactagen has been marketed as a therapy for lactose intolerance, largely based on the colonic adaptation hypothesis. Lactagen is a powder containing lactose, *L. acidophilus*, fructo-oligosaccharides, and small amounts of calcium and phosphorus. It is recommended by the manufacturer that the product be taken for a 38-day period, which is said to be sufficient to permanently "recondition" the intestinal microflora for lactose tolerance. One double-blind study of this product, published only as an abstract [147], reported that 80% of lactose-intolerant subjects had reductions in lactose intolerance symptoms compared with 19% in the placebo group. However, objective evidence of improved lactose digestion or altered colonic bacterial fermentation of lactose (e.g., decreased breath hydrogen) was not obtained in this study. Furthermore, longer term follow-up studies to assess the permanence of the symptom reductions were not conducted.

H A2 β-Casein Milk

Cow's milk typically contains A1 and A2 types of β-casein, the former of which may yield the peptide β-casomorphin-7 (BCM-7) upon digestion [148]. Adverse gastrointestinal effects such as inflammation and discomfort, which resemble lactose intolerance, have been suggested to result from A1 β-casein and its subsequent metabolite, BCM-7. Proposed effects of BCM-7 include increased gastrointestinal transit time, reduced total fecal SCFA content, and slowed cognition. Either or both of the A1 and A2 types of β-casein can be present in cow's milk depending on its genetic makeup. It has been suggested that lactose maldigesters may better tolerate cow's milk containing solely A2 β-casein. However, further evidence that A2 β-casein is associated with improved tolerance is needed.

IX GENE THERAPY FOR LACTOSE INTOLERANCE

Although conventional dietary therapies for lactose intolerance exist, the possibility of gene therapy for LNP was examined by During et al. [149]. An adeno-associated virus vector was orally administered to hypolactasic rats to increase lactase mRNA. The adeno-associated virus vector is a defective, helper-dependent virus, and the wild type is nonpathogenic in humans and other species. Following a single administration of a recombinant adeno-associated virus vector expressing β-galactosidase, all rats treated with this vector ($n = 4$) were positive for *lacZ* mRNA in the proximal intestine within 3 days. There was no lactase mRNA in the rats treated with the control vector. On day 7, following vector administration, the rats were challenged with a lactose solution. The treated rats had a rise in blood glucose from 114 ± 4 to 130 ± 3 mg/dL after 30 minutes, whereas the control rats had a flat blood glucose curve. Furthermore, the treated rats still displayed similar lactase activity when challenged with lactose 6 months later. Thus, the potential of gene therapy for lactose intolerance is an interesting prospect, but this has not been studied in humans.

X PREBIOTICS AS TREATMENT FOR LACTOSE MALDIGESTERS

Prebiotics, specifically galacto-oligosaccharides (GOS), have been shown to alter gastrointestinal microflora, which can improve lactose digestion [150]. Due to the glycosidic bonds in GOS, it is able to avoid hydrolysis by salivary and intestinal salivary enzymes. Thus, GOS reaches the colon intact, where it promotes the growth of lactose-metabolizing bacteria already existent in the digestive tract such as *Bifidobacterium* spp. and *Lactobacilli* spp. A randomized double-blind clinical trial involving 61 participants demonstrated improved lactose digestion following GOS treatment, as evidenced by fewer symptoms of lactose intolerance posttreatment. Furthermore, long-term treatment with GOS is not necessary as long as the affected bacterial colonies are continually exposed to lactose.

XI SUMMARY

A majority of the world's population and approximately 25% of the U.S. population are lactose maldigesters. Milk and milk products not only contain lactose but also are important sources of calcium, riboflavin, and high-quality protein. Some maldigesters may avoid dairy products because of the perception that intolerance symptoms will inevitably follow dairy food consumption. Avoiding dairy products may limit calcium intake and bone density, thus increasing the risk for osteoporosis. Avoidance of milk and milk products is unnecessary because moderate lactose consumption does not usually produce a symptomatic response in maldigesters. In addition, various dietary strategies effectively manage lactose intolerance by reducing or eliminating gastrointestinal symptoms. Dairy food consumption is possible for individuals who are lactose intolerant if simple dietary management strategies are incorporated into daily living.

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Chapter 41

Nutritional Considerations in the Management of Gluten-Related Disorders*

Maureen M. Leonard[1,2], Pamela Cureton[1,2] and Alessio Fasano[1,2]
[1]*MassGeneral Hospital* for *Children, Boston, MA, United States,* [2]*Harvard Medical School, Boston, MA, United States*

I INTRODUCTION

Gluten-related disorder celiac disease (also spelled coeliac disease in the European literature) has been known by many names in the medical literature in the past, including gluten-sensitive enteropathy, gliadin-sensitive enteropathy, and celiac sprue (to differentiate it from tropical sprue). Celiac disease occurs in genetically predisposed individuals who develop a permanent immunologic reaction to gluten, found in wheat, rye, and barley. When individuals with celiac disease ingest gluten, the result is malabsorption of sugars, proteins, fats, vitamins, and other minerals. Individuals with celiac disease have a permanent intolerance to the gliadin fraction of wheat protein and related alcohol-soluble proteins (called prolamins) found in rye and barley. Susceptible individuals who ingest these proteins develop an immune-mediated enteropathy, which self-perpetuates as long as these gluten-containing grains are in the diet. Removal of gluten from the diet, in the majority of people with celiac disease, leads to both resolution of symptoms and improvement in the intestinal damage. However, in some individuals, there may be a primary lack of response or secondary loss of response to the gluten-free diet (called "nonresponsive" or "refractory sprue") and intense nutritional support and immune-suppressing medications may be required.

Symptoms of celiac disease in childhood include gastrointestinal symptoms such as diarrhea, abdominal pain, or constipation [1]. Extraintestinal manifestations include short stature, anemia, dental enamel hypoplasia, and children may also present with clinically silent disease [1]. In the United States, and worldwide, many individuals are being diagnosed with celiac disease as adults [2]. They may present with gastrointestinal symptoms, such as diarrhea, gastroesophageal reflux, or constipation. Adults may also present with symptoms outside the gastrointestinal tract; including joint pain, rash, infertility, and abnormalities of the hematologic, musculoskeletal, and neurologic systems [1]. Individuals with celiac disease, and their family members, often have other autoimmune diseases, such as type 1 diabetes, inflammatory bowel disease, and thyroid disease [1]. The diagnosis of celiac disease is often delayed for years due to the presence of nonspecific complaints.

Serum antibodies are useful as a screening method for celiac disease. However, a small-intestinal biopsy, done by endoscopy, must be obtained to confirm a diagnosis of celiac disease. A patient's clinical response to a gluten-free diet is also used to provide important information about the diagnosis. Individuals with untreated disease are at risk for complications, such as iron-deficiency anemia, vitamin deficiencies, osteoporosis, infertility, and gastrointestinal cancers. The long-term maintenance of a gluten-free lifestyle is challenging. Individuals diagnosed with celiac disease benefit most from a team approach, which includes regular supervision by a physician, nutritional counseling by a dietitian, and access to support groups knowledgeable about celiac disease and the gluten-free diet.

II SYMPTOMS OF CELIAC DISEASE

The clinician must have a high index of suspicion for celiac disease because its symptoms can affect almost

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every organ system of the body, and its clinical features can be highly variable. The disease may present at any age [2]. Likewise, the severity of the presentation of celiac disease can be highly variable, with some patients experiencing severe diarrhea and weight loss, and others having no gastrointestinal symptoms whatsoever. Because celiac disease is a multisystem disease, it can affect not only the gastrointestinal tract but also the neurologic, endocrine, musculoskeletal, reproductive, and hematologic systems. Thus, it is not uncommon that a patient with celiac disease may be seen by multiple physicians over many years before the disorder is correctly identified.

A Intestinal Manifestations

1 Pediatric

Infants and toddlers may present with gastrointestinal complaints as early as 12 months of age, after the introduction of gluten-containing foods into the diet. The onset is usually insidious, with "failure to thrive" and obvious physical signs of wasting over a period of weeks to months. Symptoms include diarrhea, weight loss, abdominal distention, gassiness, and foul-smelling stools due to carbohydrate (primarily lactose) and fat malabsorption. Parents will often report that the child becomes irritable after meals, and the child may also develop a secondary anorexia due to the pain associated with the diarrhea and abdominal distention. These children exhibit classic signs on physical exam of protein-calorie malnutrition: hypotonia, poor muscle bulk, peripheral edema, decreased subcutaneous fat stores, abdominal distention, and ascites in severe cases. Anemia is common. The anemia is usually microcytic, due to iron malabsorption. However, if the disease is long standing, and small bowel involvement is severe, a macrocytic anemia can develop as a result of folate or vitamin B_{12} malabsorption. Radiographs can show osteopenia due to vitamin D and calcium malabsorption.

2 Adolescents and Adults

School-age children, adolescents, young adults, and even the elderly often complain of mild to intermittent diarrhea, gassiness, urgency, and abdominal distention and cramping. A presenting patient may also complain of chronic abdominal pain; constipation rather than diarrhea; and upper gastrointestinal tract symptoms such as nausea, dyspepsia, indigestion, gastroesophageal reflux, and chronic vomiting. Rather than being examined for celiac disease, these patients often are labeled as having irritable bowel syndrome (IBS), lactose intolerance, recurrent abdominal pain of childhood, or even an inflammatory bowel disease such as Crohn's disease. Patients are therefore treated with lactose-free or high-fiber diets, medications to treat constipation or IBS, or medications to suppress gastric acid or the immune system. Not surprisingly, the celiac patient will not get relief from these treatments. Some of these therapies, such as a high-fiber diet, which often contains a great deal of gluten, may increase symptomatology. Many patients will have upper and lower endoscopies performed by a gastroenterologist, and the results may look visually normal without obvious ulcers or cancers; thus, biopsies for celiac disease will not be taken. Because of this, the diagnosis of celiac disease is often missed.

B Extraintestinal Manifestations

Celiac disease is a multisystemic immune-mediated disorder and thus it can present with signs and symptoms outside the gastrointestinal tract [1]. Organ systems affected can include the musculoskeletal system, skin and mucous membranes, reproductive system, hematologic system, hepatic system, and central nervous system.

1 Musculoskeletal System

One of the most common presenting features in childhood in the musculoskeletal system is idiopathic short stature. In European studies [3–5], it is estimated that approximately 1 of 10 children whose height is far below his or her genetic predisposition for unclear reasons is short because of celiac disease. Short stature can be an isolated feature of this condition. The children often have delayed onset of puberty, and stimulation testing may reveal growth hormone deficiency [6]. The potential to achieve normal stature and bone mineralization is good if these patients are diagnosed with celiac disease before puberty and placed on a gluten-free diet in a timely fashion [7]. However, after the growth plates have closed, individuals will have short stature for life.

Dental enamel defects are common in children affected by celiac disease during the toddler years. They may experience caries at an early age and in atypical locations. One report has noted that up to 30% of older patients with celiac disease have enamel hypoplasia [8]. If gluten is in the diet, these enamel defects affect only the permanent (secondary) dentition that is forming before the age of 7 years. The primary dentition or "baby teeth" are formed in utero, which is a gluten-free environment, and thus the primary dentition is unaffected. The dental enamel defects are linear and occur symmetrically in all four quadrants [9]. The precise cause of these defects is unknown.

Joint pain is a common complaint among both adolescents and adults with undiagnosed celiac disease. The joints are often only painful, and not red, hot, or visibly

swollen. The pain often resolves with the implementation of a gluten-free diet. Clubbing of the fingers and toes (broad digits with abnormally curved nails) has also been reported. Interestingly, and to confuse the issue further, celiac disease is also seen in higher incidence in patients with rheumatologic disorders such as systemic lupus erythematosus and rheumatoid arthritis [10–12]. In an adolescent, arthritis may be the only presenting symptom [13].

One of the most serious causes of morbidity in older individuals diagnosed with celiac disease is fractures associated with osteoporosis. The potential health implications are grave and include vertebral fractures, kyphosis, hip fractures, and Colles' fracture of the lower radius in the arm [14]. These problems can begin in childhood, when radiographs may reveal delayed bone age, rickets, or osteomalacia, which left untreated may lead to osteoporosis. It is believed that early interventional therapy with a gluten-free diet may prevent progression and may even reverse bone loss [15]. However, severe osteoporosis associated with celiac disease diagnosed very late in life will not revert on the gluten-free diet after a critical point in time [16].

2 Skin and Mucous Membranes

Often overlooked, the skin and mucous membranes can be obvious sites for the expression of symptoms from celiac disease. The classic skin manifestation of celiac disease is a skin rash called dermatitis herpetiformis, often abbreviated as DH. It occurs primarily in adults and is rarely seen in children before puberty. This skin rash is classically pruritic (itchy) and symmetrical and does not respond to topical creams and medications. The rash begins as flat, red lesions, which then progress to erythematous, fluid-filled blisters. They usually occur on the face, elbows, back, buttocks, and knees. The lesions initially can be confused with urticaria (hives) or varicella (chickenpox). Patients will often scratch and pick at the lesions, so that the skin will then develop an eczematous appearance. A biopsy of the normal-appearing skin next to the affected area demonstrates the characteristic histology of granular immunoglobulin A (IgA) deposits [17]. DH is now known to be the pathognomonic skin rash for celiac disease, and once a skin biopsy has proven the diagnosis of DH, an intestinal biopsy for celiac disease is unnecessary. If a patient with DH does have a small-intestinal biopsy, the majority of the time there will be intestinal damage [18]. For complete resolution of DH, a gluten-free diet is required. Some patients with DH also require an antiinflammatory antibiotic, in addition to a gluten-free diet, during acute onset of DH. The skin lesions, or just the pruritis of DH, may appear when gluten is intentionally or inadvertently ingested. This should alert the affected individual to scrutinize the diet for gluten contamination.

Celiac disease can have many other manifestations in the skin and mucous membranes, including urticaria, psoriasis, and oral aphthous stomatitis [18–20]. Oral aphthous stomatitis ("fever sores" or "canker sores"), which can appear on the cheeks, tongue, gums, and lips, usually correlates with histological changes in the small intestine. However, because aphthous ulcers are also seen with common viral infections (such as herpes viruses) and other types of autoimmune and inflammatory diseases of the gastrointestinal tract (particularly Crohn's disease), they are not pathognomonic for celiac disease.

3 Reproductive System

The reproductive systems of both women and men can be affected by celiac disease. Compared to the general population, women with undiagnosed celiac disease have greater difficulty becoming pregnant [21] and a higher risk for spontaneous abortions [22,23]. Men with undiagnosed celiac disease may also experience infertility due to gonadal dysfunction [24]. Adult patients with undiagnosed celiac disease may often undergo exhaustive and expensive infertility studies, without discovery of an etiology. Many of these infertile women will also give a history of delayed menarche, anemia, and IBS symptoms, similar to the pediatric patients described previously with short stature and delayed puberty. Women with undiagnosed and diagnosed celiac disease have infants with higher rates of neural tube defects because of folic acid deficiency, as well as more infants with intrauterine growth retardation [25–27]. Whether these individuals experience reproductive challenges because of nutritional factors, or having an undiagnosed autoimmune disease, or a combination of both is unclear.

4 Hematologic System

Iron-deficiency anemia is one of the most common micronutrient deficiencies in the undiagnosed celiac patient. In addition to anemia, leukopenia (low white blood cell count) and thrombocytopenia (low platelet count) have also been reported. The anemia in celiac disease is usually microcytic and hypochromic, due to iron deficiency [28]. However, a macrocytic anemia should warrant an investigation into gut malabsorption of vitamin B_{12} or folic acid. Because fat-soluble vitamins are also malabsorbed in this disorder, vitamin K deficiency may occur, resulting in an increased risk for bruising and bleeding. Vitamin E deficiency may also occur, resulting in a hemolytic anemia and jaundice.

5 Liver

In children and adults with celiac disease, diseases of the liver, such as autoimmune hepatitis and chronic transaminasemia (liver enzymes), have been reported [28–31].

In children initially diagnosed with celiac disease with elevated aspartate transaminase and alanine transaminase liver enzymes at presentation, these biochemical markers of hepatocyte damage returned to normal with strict adherence to a gluten-free diet [32]. Autoimmune liver diseases, such as autoimmune hepatitis, sclerosing cholangitis, and primary biliary cirrhosis, are all more likely to occur in the individual with celiac disease [33]. Patients with celiac disease who have evidence of chronic liver disease that has not responded to a gluten-free diet require an evaluation for these autoimmune liver diseases.

6 Central Nervous System

There is evidence that patients with celiac disease have increased rates of both neurologic and psychiatric disorders. Their associations are not always clear: some symptoms can be attributed to nutritional deficiencies; others perhaps related to autoimmunity; and many are hypothesized to be due to yet-unproven pathways by which gluten may cross the blood–brain barrier and interact with endogenous neurotransmitter receptors or cause neuroinflammation. Neurological complications have been reported in association with established celiac disease for decades [34], and gluten sensitivity can present with neurological dysfunction as its sole symptom [35].

In the neurologic system, patients with celiac disease are 20 times more likely than the general population to have epilepsy and have been reported with associated cerebral and cerebellar calcifications imaged by both computed tomography and magnetic resonance imaging [36]. In children with celiac disease, focal white matter lesions in the brain have been reported and are thought to be either ischemic in origin as a result of vasculitis or caused by inflammatory demyelination [37].

Gluten-associated ataxia is the most common neurological manifestation of gluten sensitivity [38] and presents as difficulty with speech, movement, and balance due to atrophy of the cerebellum. It rarely occurs before puberty, has a mean age of onset in the late 40s, and has a paucity of gastrointestinal symptoms [39]. Emerging evidence indicates that early initiation of a gluten-free diet is beneficial for treating the symptoms of both ataxia and peripheral neuropathy, even in the absence of intestinal damage [40].

In both treated and untreated persons with celiac disease, psychiatric comorbidities, such as depression, dementia, and schizophrenia, are common. In children, behavioral changes such as irritability, increased separation anxiety, emotional withdrawal, and autistic behaviors have improved, by parental report, on a gluten-free diet [41]. Although not scientifically validated, the gluten-free diet, along with a casein-free diet, is now also being advocated by several groups for a subset of children with autism [42–44]. Children with Down syndrome and other genetic syndromes are at higher risk for celiac disease [45]. It is important to note that only a proportion of patients presenting with neurological dysfunction associated with gluten sensitivity will also have biopsy-proven intestinal damage [46].

C Associated Conditions

There are several groups of patients who are at substantially increased risk of having celiac disease: those with another autoimmune disorder, those with certain syndromes, and relatives of biopsy-diagnosed patients. These associated conditions are further described in Table 41.1. Patients with other autoimmune diseases, such as type 1 diabetes, as well as relatives of persons with biopsy-diagnosed celiac disease, are more likely to carry the human leukocyte antigen (HLA) haplotypes that put them at risk for the disease. It has been demonstrated that patients diagnosed with celiac disease early in life, and treated early with a gluten-free diet, have a significantly lower risk of developing other autoimmune disorders than do individuals diagnosed later in life [71].

Persons with both Down and Turner syndromes classically have short stature and are at increased risk for skeletal and other autoimmune diseases such as diabetes mellitus, Crohn's disease, and thyroid disease [72], which makes the diagnosis in this population exceptionally difficult. A multicenter study from Italy strongly suggested the need for screening all children with Down syndrome, regardless of the presence or absence of gastrointestinal symptomatology [58]. It is unclear why individuals with certain syndromes are at higher risk for celiac disease. However, because several of these syndromes have as part of their constellation poor growth and short stature, anthropometric measures are poor screening tools for malabsorption in these populations. The NIH Consensus Development Conference on Celiac Disease, held in June 2004, recommended that patients with Down, Turner, or Williams syndrome should be offered screening for celiac disease at least once [73].

D Asymptomatic Disease

It can be debated whether or not there is truly an "asymptomatic" form of celiac disease. The medical literature describes patients lacking the signs and symptoms occurring in the gastrointestinal tract or extraintestinal systems as "asymptomatic." With a more detailed history, physical exam, and laboratory investigations, these asymptomatic patients may have revealed evidence of trace mineral deficiencies, anemia, short stature, low bone density, and low serum fat-soluble vitamin levels. These patients may be identified through mass serologic screenings and are

TABLE 41.1 Conditions and Syndromes at Increased Risk for Celiac Disease

Condition	% Diagnosed with Celiac Disease	Increased Risk	Reference
Arthritis	1.5–7.5	3- to 10-fold	[1,10,47,48]
Cardiomyopathy (idiopathic dilated)	5.7		[49]
Dental enamel defects	19–30	19-fold	[8,9,50]
Dermatitis herpetiformis	100		[17,51,52]
Diabetes type 1	3.5–10	4- to 10-fold	[53–57]
Down syndrome	4–20	17- to 50-fold	[1,58–60]
Epilepsy	2		[36,61]
IgA deficiency	7	31-fold	[62]
Infertility (idiopathic)	6.3		[1]
Iron-deficiency anemia	4.2	16-fold	[1,28]
Osteoporosis	2.6		[1]
Primary biliary cirrhosis	6		[33]
Relatives, first- or second-degree	2.6–20	18-fold	[1,63,64]
Short stature (idiopathic)	4–10	23-fold	[1,3–5]
Sjögren's syndrome	2–3		[1,65]
Thyroid disease (autoimmune)	4		[66]
Turner syndrome	4–8		[67–69]
Williams syndrome	9.5		[70]

often first- and second-degree relatives of a patient with biopsy-diagnosed disease. A family history of gastrointestinal cancers or other autoimmune disorders is often elicited [74–77].

E Potential Celiac Disease

Patients with the potential celiac disease have positive antibodies for the condition but an initial normal intestinal biopsy. The evidence regarding the likelihood of developing celiac disease and factors useful in predicting whether overt celiac disease develops is still debated. Pediatric studies report that the majority of patients with potential celiac disease do not go on to develop overt celiac disease; however, factors predictive of developing the disease have not been identified [78]. A prospective study examining 175 asymptomatic children with potential celiac disease with serology tests and intestinal biopsies every 2 years found that at 3, 6, and 9 years of follow-up, the number of cases that remained potential were 86%, 73%, and 67%, respectively [79]. While the true prevalence of potential celiac disease and the frequency with which it develops into overt celiac disease are unknown, studies indicate the prevalence, like overt celiac disease, is rising [80].

III DIAGNOSIS OF CELIAC DISEASE

A Serologic and Genetic Screening Tests

The tests of choice to screen for celiac disease are serum immunological markers with high sensitivity and specificity. The classic screening tests that are used for other gastrointestinal malabsorption disorders, such as fecal fat, glucose tolerance tests, D-xylose uptake, serum carotene levels, and permeability tests (e.g., lactulose/mannitol ratios), are poorly specific for celiac disease and should not be used in place of the serologic markers that are discussed hereafter. The clinician must remember, however, that no screening test is perfect, and that the "gold standard" to confirm the diagnosis of celiac disease remains a small-intestinal biopsy and the patient's clinical response to a gluten-free diet. Any patient with suggestive symptoms should have a small-bowel biopsy, even if the serology is negative.

There are three frequently used commercially available serologic tests (antibodies): anti-endomysial or

anti-endomysium (EMA or AEA), antitissue transglutaminase (tTG), and antideamidated gliadin antibody (DGP). Sensitivity, specificity, and positive and negative predictive values can vary widely for each antibody, depending on the age of the patient, the population being studied, the substrates and laboratory kits being used to run the assays, and the proficiency of the laboratory performing the test [81]. Therefore, the IgA tTG is the first line screening test and the EMA is sometimes used to confirm the tTG findings before moving forward with endoscopy. Conditions that may yield false negative antibody results include a patient who makes low levels of IgA, young children who may not manufacture autoimmune antibodies, an inexperienced laboratory, and testing while the patient is already on a gluten-free diet. DGP antibodies of IgG class have been introduced with a sensitivity and specificity close to IgA anti-tTG. The DGP test is recommended in limited circumstances most notably, the immunoglobulin G (IgG) DGP antibody test is recommended for use when IgA is low (<7). False positive tests can also be seen with these antibody tests, as delineated later, in normal individuals with other gastrointestinal disorders and in other autoimmune disorders.

1 Anti-Endomysium Antibodies

The serologic test currently commercially available with the highest sensitivity and specificity is the anti-endomysium IgA immunofluorescent antibody (EMA). The EMA was discovered in the early 1980s and rapidly gained use as part of a screening "celiac panel" by commercial laboratories in combination with AGA IgG and AGA IgA. False-negative EMA can be seen in young children, those with IgA deficiency, and in the hands of an inexperienced laboratory because of the subjective nature of the test [81]. Also, the substrate for this antibody was initially monkey esophagus, making it expensive and unsuitable for screening large numbers of people. Human umbilical cord is now used as an alternative to monkey esophagus in most commercial laboratories [82].

2 Tissue Transglutaminase Antibodies

Tissue transglutaminase (tTG) was described in 1997 as the autoantigen of EMA [83]. The initial tTG ELISA was guinea pig IgA, with a lower sensitivity and specificity than EMA [84,85]. However, commercial labs now use human recombinant tTG, which has improved sensitivity and specificity and correlates better with EMA IgA and intestinal biopsy results [83–86]. The tTG IgA ELISA represents an improvement over the EMA IgA assay because it is less expensive, is less time-consuming, is not a subjective test, and can be performed on a single drop of blood using a dot-blot technique, making this an ideal test for mass serologic screenings. Positive tTG results may be seen in other autoimmune diseases, such as type 1 diabetes, autoimmune liver disease, autoimmune thyroid disease, and inflammatory bowel disease.

3 Genetic Testing

Although celiac disease is the only autoimmune disease for which we know the environmental trigger, gluten, we also know that there is a strong genetic influence. For example, as discussed under Section II-C, Associated Conditions, in first- and second-degree relatives, there can be up to a 26% disease prevalence [1,63,64,80]. Also, identical twins have a 75% concordance rate for celiac disease (one of the highest rates reported for any disease), whereas nonidentical twins do not differ from siblings [87]. This again indicates a genetic, in addition to an environmental, component.

Celiac disease is a complex genetic disorder, but the actual "celiac genes" have not yet been identified. The strongest genetic determinant of risk for celiac disease appears to be the presence of certain HLA alleles. The presence of these HLA alleles, DQ2 and DQ8, is thought to account for up to 40% of the genetic load of the familial risk for celiac disease [88]. The HLA are markers that help identify cells as "self" versus "nonself." The HLA prevent the immune system from attacking "self." HLA DQ2 or DQ8 are found in more than 95% of celiac patients. It is extremely rare for a celiac patient to have neither of these genes. However, if an individual has these HLA alleles, it does not mean that the individual has celiac disease because these alleles are found in 39.5% of the general population [89–91]. This is a great source of confusion for patients and physicians because it is counterintuitive to other types of genetic testing, where the presence of the gene confirms the disease.

The value of genetic testing is that it has a high negative predictive value to rule out celiac disease for a patient's lifetime. Negativity for HLA DQ2 and DQ8 excludes the diagnosis of celiac disease with 99% confidence. However, positivity for DQ2 or DQ8 has limited diagnostic value because of the high prevalence of these genotypes in the general population. The strength of genetic testing is that the patient does not need to be on a gluten-containing diet to be tested because the presence of these genes is not affected by diet. Therefore, genetic testing can evaluate

- Infants not yet exposed to gluten;
- Young children who may not make all of the antibodies;
- Patients who have self-imposed a gluten-free diet;
- Patients with serology or biopsies that were not conclusive;
- Relatives of biopsy-diagnosed individuals with celiac disease.

4 Current Guidelines on the Use of Serologic and Genetic Testing to Screen for Celiac Disease

As described previously, the IgA class human anti-tTG antibody, coupled with a determination of total serum IgA to rule out deficiency, currently is the most cost-effective way to screen for celiac disease in an otherwise healthy adult. EMA should be used as a confirmatory, prebiopsy test, whereas IgG DGP determinations should be restricted to the diagnostic workup of individuals with IgA deficiency. Given the high prevalence of the HLA haplotypes associated with celiac disease in the general population, genetic testing is not recommended as a routine screening test for celiac disease. Additionally, most insurance companies will not cover the test making it quite expensive. The practitioner should remember that serologic tests are screens and that to confirm the diagnosis of celiac disease, a small bowel biopsy must be performed, as discussed next.

B The Intestinal Biopsy

Confirmation of either a clinical suspicion of celiac disease or a positive serologic screen requires a small-intestinal biopsy. Before the advent of fiberoptic and chip technology for endoscopes, biopsies of the jejunum were obtained using a Crosby spring-loaded capsule, passed orally under fluoroscopic guidance. Biopsies today are performed using a flexible endoscope, passed orally under either conscious sedation or general anesthesia. This has the advantages of allowing direct visualization of the mucosa with a camera to look for changes suggestive of small bowel damage, such as notching or scalloping of the small bowel folds or lymphonodular hyperplasia [92]. Endoscopy also allows the endoscopist to look for other lesions, such as ulcers, esophagitis, or gastritis, which may help explain the patient's symptomatology.

The detailed description of the characteristic small bowel changes seen in celiac disease that was given by Marsh in 1988 has become accepted as the standard [93]. The Marsh criteria describe four patterns of mucosal pathology: type 0 (preinfiltrative), which is without detectable inflammation or changes in the crypt/villous architecture; type 1 (infiltrative), with an increase in the intraepithelial lymphocytes (IELs) but without detectable changes in the crypt/villous architecture; type 2 (hyperplastic), with inflammation, villous blunting, and an increased crypt/villous height ratio; and type 3 (destructive), with severe inflammation, flat villi, and hyperplastic crypts. The clinician, however, needs to be aware that villous atrophy (shortening of the finger-like projections in the small bowel, which increase absorptive surface area) can be caused by a wide variety of gastrointestinal diseases and infections, and that correlation with serology and the patient's response to a gluten-free diet is imperative to confirm the diagnosis. In rare instances, a gluten challenge may be necessary to confirm that the villous atrophy was due to celiac disease and not a concomitant gastrointestinal infection. In cases in which serology is suggestive, the biopsy is confirmative, and the patient has had a clinical response to the gluten-free diet, a gluten challenge and repeat small bowel biopsy are no longer required [94]. However, a repeat small bowel biopsy in order to confirm intestinal mucosal healing should be considered in pediatric and adult cases.

IV TREATMENT OF CELIAC DISEASE WITH A GLUTEN-FREE DIET

The only known treatment for celiac disease is a gluten-free diet. This was first discovered after World War II in children with celiac disease when the toxicity of wheat proteins was established after the bread shortages resolved in Europe [95,96]. Gluten is important in baked goods because it plays an important role in leavening, in forming the structure of the dough, and in holding the baked product together [97]. Removal of gluten from the diet of a biopsy-diagnosed person with celiac disease results in complete symptomatic and histologic resolution of the disease in the majority of patients. The identified agents responsible for the immune-mediated response and intestinal damage are prolamins, storage proteins located in the seeds of different grains. Gluten is the general name for the prolamins found in wheat (gliadin), rye (secalin), barley (hordein), and oats (avenin) [97].

The prolamin of oats, avenin, accounts for only 5–15% of the total seed protein, as opposed to gliadin, which comprises approximately 50% of wheat proteins [98]. This oat prolamin is thought not to elicit the same immune response as gliadin and is thought by some to be safe for patients with celiac disease to ingest [51]. The risk that oats are contaminated with wheat in the United States is great because oats are often crop-rotated, harvested, and milled with wheat. For this reason, patients are instructed to use only labeled gluten-free oats when including oats in their diet. A study in the United States in newly diagnosed children with celiac disease who were allowed to eat oats found that these children had symptomatic and histologic resolution of the disease comparable to children who were denied oats [99]. Prolamins are also found in corn and rice, but they likewise do not elicit an immune reaction in the intestines of individuals with celiac disease [97].

Table 41.2 provides some basic dietary guidelines for persons following a gluten-free diet. Although not all-inclusive, it is meant to serve as a starting point for

TABLE 41.2 Basic Dietary Guidelines for Individuals Following a Gluten-Free Diet

Allowed	Not Allowed	Questionable
Fruits and Vegetables		
Grains/Starches: corn (maize), corn bran, corn starch, corn meal, corn flour, corn germ, corn gluten, rice, rice bran, rice flour, glutinous white rice, rice polish, soy (soya), arrowroot, pure wild rice, sago, potato starch, potato flour, sweet potato flour, legume flours (garbanzo, chickpea, garfava, lentil, pea, whole bean), nut flours (almond, chestnut, hazelnut), flax seed, flax seed meal, sorghum, tapioca (also called cassava or manioc), buckwheat, millet, teff, amaranth, quinoa	Grains: wheat, rye, triticale, barley, wheat flour, wheat germ, wheat bran, graham flour, gluten flour, durum flour, wheat starch[a], bulgur, farina, semolina, couscous, spelt, kamut, einkorn, emmer, farro, orzo, atta Oats (in any form) unless uncontaminated and labeled gluten-free oats	Packaged rice mixes Rice pilaf-may contain Italian vermicelli (a wheat-based pasta) and other gluten-based ingredients Buckwheat flour (pure buckwheat is gluten-free but buckwheat flour may be a blend of buckwheat and wheat)
Meat/Protein Foods		
Fresh, plain, frozen, and smoked meat (beef, pork), poultry, fish, seafood without added, unidentified natural flavorings or seasonings Veggie burgers and meat substitutes that are labeled gluten-free and do not contain any questionable ingredients Processed meat or poultry products (such as deli or luncheon meats) labeled gluten-free or free of gluten-containing ingredients Fresh eggs (in the shell) Most cholesterol-reduced liquid egg products Lentil, chickpea (garbanzo), dried peas, soybean, garfava (garbanzo and fava), whole dried beans (navy, pinto, black) Most peanut and nut butters Plain tofu Plain soy miso, rice miso Plain nuts (almond, walnut, chestnut, hazelnut, etc.) Plain peanuts Plain seeds (sesame, sunflower, chia, pumpkin, hemp, flax) Tempeh (made without gluten-containing ingredients)	Canned fish, poultry, or meat basted containing hydrolyzed wheat protein Most veggie burgers and many vegetarian meat substitutes—most contain gluten in the form of soy sauce made from wheat, texturized wheat protein, seitan, wheat gluten, bulgur wheat, wheat flour, or oats Roast beef or prime rib with au jus (many beef sauces/gravies contain hydrolyzed wheat or wheat flour) Processed, prepared, or preserved meat and meat products (luncheon meats, hot dogs, sausages, etc.), processed poultry products (seasoned chicken breast, etc.), and processed mixed food products (that generally contain more than 3% raw meat or 2% or more cooked meat or poultry meat) that contain modified food starch, dextrin and/or starch derived from gluten or other gluten-containing ingredients are present Processed egg products (dried, frozen, or liquid eggs with or without added ingredients) that contain modified food starch, dextrin, and/or starch derived from gluten	Any meat, poultry, or fish product containing unidentified natural flavoring or seasonings (beef, fish, or chicken burgers may contain fillers such as wheat flour, wheat starch, bread crumbs) Seasonings may include ingredients derived from wheat, barley, or rye Ham (ready to cook)—glaze may contain wheat protein, wheat flour, or wheat starch Poultry, fish, or meat marinades—may be made with soy sauce, malt vinegar, or other gluten-containing ingredients Surimi (imitation crab/seafood) Imitation bacon Reduced fat and flavored peanut and nut butters—check for gluten-containing ingredients Flavored/seasoned tofu—may be made with soy sauce or other gluten-based ingredients Miso (may be barley based)
Milk Products		
Milk: whole, low fat, skim, plain powdered, evaporated, or condensed Soy, hemp, nut (almond), coconut, rice, and sunflower seed-based milk substitutes labeled gluten-free	Malted milk Yogurt with gluten-based mix-ins (cookie crumbs, granola, etc.)	Soy, hemp, nut (almond), coconut, and rice-based milk substitutes—may contain barley flavoring and/or barley-derived enzymes Chocolate drinks and mixes—may contain barley malt or other gluten-containing ingredients
Buttermilk		
Cream, half & half, nondairy creamer Regular sour cream Plain yogurt		
Fruits and Vegetables		
All plain fresh, frozen, and canned fruits Pure fruit juices—all plain fresh, frozen, dried, and canned vegetables Pure vegetable juices Plain tomato sauce	Breaded or batter-dipped vegetables, tempura, vegetables with sauces containing wheat-based soy sauce, teriyaki sauce, or unknown ingredients Frozen potato products made with wheat starch or flour	Canned fruit pie filling—may contain gluten-containing ingredients Dried fruit (esp. dates)—may be dusted with flour or starch to prevent sticking Bulk bin dried fruit—may be contaminated with items from other bins

(*Continued*)

TABLE 41.2 (Continued)

Allowed	Not Allowed	Questionable
Fruits and Vegetables		
Plain tomato paste Spaghetti sauce made with allowed ingredients Fresh potatoes Gluten-free French fries cooked in dedicated fryer		French fries—"seasoned" or flavored fries—often contain wheat starch or wheat flour Restaurant/fast food fries—often cooked in oil used to cook gluten-containing foods (i.e., onion rings, breaded chicken fingers) Scalloped potatoes are usually made with flour Potato mixes may contain gluten-based ingredients
Miscellaneous		
Butter, margarine, shortening, all vegetable oils (olive, sunflower, safflower, canola, grape seed, etc.) except wheat germ oil Most ice cream and frozen yogurt, sherbet, sorbet, popsicles, gelatin, Italian ice, whipped cream Pure chocolate, honey, maple syrup, jelly, jam, marmalade, molasses, sugar (white and brown), coconut, fructose, powdered/confectioner's sugar (if corn starch based), artificial sweeteners, pure cocoa powder, pure carob chips and pure carob powder, marshmallows, most chocolate syrups, some rice syrups Most relish, ketchup, prepared mustards, olives, salsa Many salad dressings Mayonnaise Most vinegars (except malt vinegar) All pure spices and herbs, pure black pepper, mustard flour (ground mustard seeds), tahini Soy sauce and marinades made without wheat or gluten-containing ingredients (labeled gluten-free) Salt MSG (monosodium glutamate) Pure or artificial vanilla extract Baking soda, yeast (except Brewer's yeast), most baking powder, cream of tartar, corn starch Corn gluten, corn malt Gluten-free Communion wafers Gums: xanthan, guar Pure tea, most herbal tea, unflavored coffee (instant or ground), pure cocoa powder, soft drinks, cider (without gluten-containing ingredients) Most distilled alcoholic beverages (i.e., rum, whiskey, vodka) without added gluten-containing flavorings Wine Water Most hot chocolate mixes, most liqueur, some soy beverages Beer labeled gluten-free and made from gluten-free grains (such as sorghum)	Wheat germ oil, nonstick baking spray containing flour, fats/oils that have been used to cook gluten-containing foods Ice cream or frozen yogurt containing gluten-based ingredients, and/or mix-ins made from gluten-based ingredients (such as cookie crumbs) Candies, candy bars, and chewing gum containing malt flavoring or other gluten-containing ingredients Licorice candy unless labeled gluten-free Soy sauce made from wheat Teriyaki sauce, Tamari, or shoyu made from wheat Malt vinegar Sauces, marinades, or gravies made with wheat flour, wheat starch, or other unknown ingredients Salad dressings containing wheat-based soy sauce, malt vinegar, or other gluten-containing ingredients Salsa containing malt vinegar or other gluten-containing ingredients Barley malt, barley flavoring, malt flavoring, or malt extract Regular Communion wafers Brewer's yeast Autolyzed yeast and autolyzed yeast extract unless the source is identified as gluten-free or the product is labeled gluten-free Malted beverages, beer, ale, lager Herb tea containing roasted barley, barley malt Postum or other grain-based coffee substitutes Beer made with gluten-containing grains—"processed or treated or crafted to remove gluten." *Note*: The gluten content of these products cannot be verified	Pudding, custards, flan, lemon curd (homemade or from mix)—may be made with flour "Flourless" cakes—may be baked in pans dusted with flour Chocolate with crisped rice—may contain malt flavor Chocolate bars may contain barley malt extract/flavoring, wheat starch, or wheat flour Butterscotch morsels—may contain barley flavoring Confectioner's sugar—may contain wheat as an ingredient Spice blends or seasoning mixes (i.e., taco seasoning mix)—may contain wheat starch or hydrolyzed wheat protein Steak sauce, Worcestershire sauce, BBQ sauce, and marinades (poultry, fish, or meat)—often contain malt vinegar, wheat-based soy sauce or other Salad dressing—may contain wheat-based soy sauce or gluten-based ingredients Prepared mustards—some made with wheat flour Restaurant sauces, gravies—most thickened with flour or use flavoring ingredients containing gluten Flavored or herb tea—may contain barley Flavored coffee—may contain gluten-containing ingredients Chocolate drinks and mixes, coffee flavored mixes—may contain barley malt or other gluten-based ingredients Alcoholic beverages containing unknown flavorings added after distillation

[a]*Except in labeled gluten-free foods.*

Source: This table has been modified with permission from The Celiac Center and Nutrition Services at Beth Israel Deaconess Medical Center.

discussion between patients and health care practitioners. Many newly diagnosed individuals are not aware that "gluten-free" does not mean just eliminating bread and pastries from the diet, because gluten (especially wheat) can be identified on food labels and in restaurants by many other names. For example, triticale (a combination of wheat and rye), kamut, and spelt are all forms of wheat and are considered toxic [100]. Other forms of wheat, such as bulgur, couscous, einkorn, farina, and semolina (durum), are also not permitted on the gluten-free diet. Any food product that contains rye, barley, or malt (a partial hydrolysate of barley) has prolamins that are considered harmful [101]. In general, a food product that includes wheat in its name (e.g., cracked wheat, wheat bran, wheat grass, wheat germ, or whole wheat) or malt in its name (barley malt, malt extract, malt flavoring, or malt syrup) is considered to contain gluten. One notable exception, however, is buckwheat, which is not directly related to *Triticum* and is considered safe to consume. Distilled ingredients (e.g., vinegar and alcohol) are allowed because gluten does not pass into the distillate. Malt vinegar is a fermented product and must be avoided as well as beverages made with barley (e.g., beer, ale, lager, and some rice and soy drinks) [97].

Food labeling in the United States has undergone some changes. The Food Allergen Labeling and Consumer Protection Act (FALCPA) was signed into law in August 2004. It requires food labels to clearly state if a product contains any of the top eight food allergens: milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, soybeans, and wheat. All food products manufactured and sold in the United States after January 1, 2006, are required to have updated labels declaring the presence of any of the top eight food allergens in the product. FALCPA was primarily passed to benefit individuals with food allergies. However, it is also of tremendous value to those with celiac disease because wheat is often hidden on ingredient labels as "modified food starch," "flavorings," "seasonings," or "dextrin" (Table 41.2). Wheat is often used as a flavoring in candy, sauces, seasonings, soups, and salad dressings [97,102]. Because wheat is the most commonly used grain in the United States, by clarifying the source of ingredients and identifying "wheat," approximately 90% of labeling concerns are resolved for celiac and gluten-sensitive patients.

The law also called for the Food and Drug Administration (FDA) to issue rules, by 2008, detailing what it means when a product is labeled "gluten-free." Unlike Europe, Canada, and Australia, the United States did not have a defined standard for "gluten-free" foods, causing a great deal of confusion over what it means when an American manufacturer puts "gluten-free" on a product's label. The FDA rule establishing the definition of "gluten-free" was finally passed and was enacted as of August 2013. The rule establishes a standard that will increase consumer confidence in the safety of products labeled gluten-free. A summary of the FDA gluten-free label rules enacted in August 2013 include:

- A food label gluten-free must:
  - Be inherently gluten-free (raw vegetables, water, 100% juice);
  - Does not contain an ingredient that is a gluten-containing grain such as wheat, rye, barley;
  - Does not contain an ingredient derived from a gluten-containing grain that has not been processed to remove gluten;
  - May contain an ingredient derived from a gluten-containing grain that has been processed to remove gluten (wheat starch) as long as the food does not contain more than 20 ppm gluten;
  - The food product contains less than 20 ppm gluten.
- Applies to foods that are regulated by the FDA:
  - Does not cover pet food, cosmetics, drugs, foods regulated by the USDA and beverages regulated by Alcohol Tobacco Tax and Trade Bureau.

Manufactures are not required to test either the ingredients or the end product.

Toiletries, such as shampoos, conditioners, and skin care products, are thought not harmful as long as they are not ingested. Patients with open skin lesions that cover a large body surface area, such as with DH, may have reactions on the skin or systemically if gluten gets into an open wound.

In addition to carefully selecting foods that are gluten-free, one must also protect these foods from coming in contact with gluten-containing foods to prevent cross contact. Examples of problematic cross contact would include, taking the bun off the hamburger patty, eating the toppings from a gluten-containing pizza, using the same toaster for both gluten-free and gluten-containing bread products, and French fries made in the same oil as other breaded products such as chicken nuggets.

The education of the person newly diagnosed with celiac disease should consist of a team approach between the patient (or parents), the gastroenterologist, the primary care physician, the dietitian, and local branches of support groups. Medical management primarily consists of monitoring for compliance with the gluten-free diet, confirming mucosal recovery, and screening for the well-known complications to be discussed later. After the gastroenterologist who performed the biopsy confirms the diagnosis, the patient should be immediately referred to a knowledgeable dietitian for medical nutrition therapy [103]. Physicians and dietitians should encourage the patient to join support organizations, which can aid in finding local resources, such as supermarkets, food manufacturers,

literature, and restaurants, that are familiar with the gluten-free diet.

Lifelong compliance with the gluten-free diet is challenging. Numerous barriers face those following a strict gluten-free diet, including availability of gluten-free foods, taste and quality of the safe foods, and the high cost of specialty gluten-free items. These products can be 3–5 times more expensive than their gluten-containing counterparts. The most important factors in achieving compliance are patient education, close supervision by an interested physician, and regular nutritional counseling by a registered dietitian with expertise in this area [103]. Compliance can be improved even in adolescents if they are seen by a physician on a regular basis [104,105]. One of the best and least expensive markers for dietary compliance is assessment by a trained interviewer (either a physician or a dietitian) because of the low cost and noninvasive nature of dietary assessment. There is a strong correlation between self-reported intake of foods containing gluten and intestinal damage [105].

V MANAGEMENT OF THE COMPLICATIONS OF CELIAC DISEASE

Patients with undiagnosed and untreated celiac disease as well as those with nonresponsive or refractory celiac disease have increased morbidity and mortality due to associated conditions, including osteoporosis, nutritional deficiencies, extraintestinal manifestations, other autoimmune diseases, and cancer. These patients also incur increased health care costs because of being chronically ill, the need to see multiple subspecialists, and the tests performed on them until the correct diagnosis is obtained [106]. Corrao and other investigators of the Club del Tenue Study Group formed a prospective cohort study that included 1072 adults with diagnosed celiac disease and 3384 first-degree relatives. These individuals were followed for 32 years. The number of deaths between the two groups was compared and expressed as the standardized mortality ratio (SMR) and relative survival ratio. Two times the number of persons with celiac disease died compared to the relatives (SMR, 2.0; 95% confidence interval, 1.5–2.7). The greatest excess of deaths occurred during the first 3 years after diagnosis. These results suggest that prompt and strict dietary treatment may decrease premature mortality among persons with celiac disease [107].

The primary reason for the increased mortality is the association with gastrointestinal malignancies, primarily intestinal lymphoma, which has been reported in up to 10–15% of adult patients who have been noncompliant with the diet [108]. The odds ratio overall for non-Hodgkin's lymphoma associated with celiac disease compared to first-degree relatives was reported to be 3.1, with odds ratios of 16.9 for gut lymphoma and 19.2 for T cell lymphoma, respectively [109]. The good news is that the reported risk for lymphoma decreases to that of the general population on the gluten-free diet [110].

A Nonresponsive and Refractory Celiac Disease

The majority of individuals with celiac disease will have a substantial improvement within the first weeks or months of being on a gluten-free diet; however, 7–30% of celiac disease patients may continue to have symptoms and intestinal damage despite being on a gluten-free diet [111,112]. Nonresponsive or refractory celiac disease is defined as persistent or recurrent signs or symptoms and/or persistent villous atrophy, despite maintenance of a strict gluten-free diet for 1 year. This may be a primary nonresponse to a gluten-free diet meaning someone who initially fails to respond to a gluten-free diet or a secondary loss of response or a relapse in symptoms after an initial improvement. The most common cause of persistent symptoms in patients with celiac disease is largely caused by inadvertent gluten exposure accounting for up to 50% of the nonresponsive celiac disease [113,114]. Patients with celiac disease may be unintentionally exposed to up to 2 g of gluten per day. Therefore, given the challenges of a gluten-free diet, a thorough dietary history should first be taken to exclude inadvertent (or intentional) ingestion of gluten. In any celiac patient with persistent symptoms, one should refer to the gastroenterologist for a repeat intestinal biopsy [115]. The gastroenterologist will also consider other common causes of associated symptoms such as IBS, other food intolerances like lactose or fructose, small-intestinal bacterial overgrowth in addition to nonresponsive or refractory celiac disease. Additional reasons for nonresponsiveness to the gluten-free diet include pancreatic insufficiency and T cell lymphoma, both of which may be associated with celiac disease [116,117].

1 Nonresponsive Celiac Disease

Patients with nonresponsive celiac disease may present at any age and typically have gastrointestinal or extraintestinal manifestations or they may be clinically asymptomatic and still have persistent intestinal damage. Patients with persistent enteropathy despite maintaining a gluten-free diet may respond to a gluten-free diet free from processed foods called the gluten contamination elimination diet [118]. The goal of this diet, followed for 3–6 months with the guidance of a dietitian is to eliminate exposure to any possible source of gluten cross contamination from packaged or processed foods, including those labeled

gluten-free. A retrospective study which evaluated 17 patients with nonresponsive celiac disease found that after approximately 4.3 months on this diet 81% of patients had resolution of symptoms, 82% had a serological or histological improvement, and 79% were able to successfully return to a traditional gluten-free diet [118]. Patients with persistent enteropathy which does not improve after 3–6 months on the gluten contamination elimination diet (as measured during repeat endoscopy) are diagnosed with refractory celiac disease.

2 Refractory Celiac Disease

Persistent villous atrophy, despite maintenance of a strict gluten free diet for 1 year after possible gluten contamination or other causes of villous atrophy have been excluded, is defined as refractory celiac disease. Refractory celiac disease is further divided into type one in which the IELs carry a normal phenotype, and type two in which there is an abnormal clonal expansion of IELs that lack surface CD3, CD8, and T cell receptors [119]. Patients with type 1 refractory celiac disease may present at any age with symptoms such as diarrhea, abdominal pain, fatigue, and others. These patients do not respond to the gluten contamination elimination diet and instead frequently require immunosuppressing medications, such as steroids including budesonide, azathioprine, and cyclosporine [111,120–124], in addition to a gluten-free diet. Despite the need for medication in addition to a gluten-free diet, patients have a 5-year survival of nearly 100% [125].

Patients with refractory celiac disease type two have a severe presentation compared to those with type one. Typically, patients present after the age of 50 years with severe diarrhea and weight loss that often requires hospitalization and support with parenteral nutrition [126]. Patients with refractory celiac disease type two are likely to be homozygous for DQ2 and often present in the fifth decade with severe protein-losing enteropathy that is unresponsive to medical management [127]. Patients with refractory celiac disease type two have a 5-year survival of 44–58% as up to half may go on to develop enteropathy-associated T cell lymphoma [119,128]. Patients respond poorly to available treatment options such as Remicade, cladiribine, and other immunosuppressant agents [129,130].

B Long-Term Monitoring

All patients with celiac disease are at risk for nutritional deficiencies. Children should be examined for protein-calorie malnutrition, linear growth failure, and delayed puberty. All patients should be screened at diagnosis for the nutritional deficiencies that can accompany this malabsorptive disorder, such as iron deficiency anemia, B_{12} deficiency, and 25-hydroxy-D. Patients should also be monitored yearly once stable on a gluten-free diet by a knowledgeable gastroenterologist for common extraintestinal complications, including osteoporosis, neurologic complaints, and the development of other autoimmune diseases, especially of the thyroid and liver [131,132]. Bone density should be measured in the newly diagnosed celiac patient because numerous studies have documented low bone density in both children and adults at the time of initial diagnosis of celiac disease. Osteopenia can improve with the gluten-free diet, and progression of osteoporosis can be halted with strict adherence to the diet and, when necessary, appropriate supplementation [133–136]. Osteopenic patients should be evaluated for deficient intake and absorption of vitamin D and calcium and the development of secondary hyperparathyroidism [137].

1 Type 1 Diabetes and Celiac Disease

Special considerations need to be given to patients who have both celiac disease and type 1 diabetes because many of the well-known complications of type 1 diabetes can be exacerbated by nutritional deficiencies. Nocturnal hypoglycemia with seizures and recurrent, unexplained hypoglycemia with a reduction in insulin requirements should prompt the physician to investigate for celiac disease [138,139]. In the young child, growth failure and delayed sexual maturation may be seen. Vitamin A deficiency may aggravate retinopathy; deficiencies of vitamins E and B_{12} may cause peripheral neuropathy; iron and folic acid deficiencies may lead to complications of fertility and pregnancy; and vitamin D can complicate dental disease, limit joint mobility, and cause osteopenia and osteoporosis. There is also an increased incidence of other autoimmune diseases in type 1 diabetics who have celiac disease [140,141]. The gluten-free diet presents additional challenges to the diabetic patient, who may see acute hyperglycemia and a steady rise in hemoglobin A1c on initiation of this diet. This can be due to intestinal healing and better absorption, as well as gluten-free food substitutes, which can be corn-, rice-, or potato-based and have a higher glycemic index.

Once the patient has undergone initial counseling, the gastroenterologist and dietitian should follow up with the patient in 3 months and again at 6 months to discuss compliance with the diet and reinforce its importance. The gastroenterologist should repeat the screening laboratory study (IgA tTG) at 6 months and again 12 months after the initial diagnosis of celiac disease. After 1 year on a gluten-free diet, a repeat endoscopy with biopsy to assess for mucosal recovery should be considered in patients with celiac disease. Once mucosal recovery is confirmed in patients with celiac disease they should be followed

annually by a gastroenterologist. At the annual visit, a detailed dietary history should be elicited, and serum antibodies can be measured. First-degree relatives should be offered serologic screening. The gastroenterologist will perform a detailed history and physical aimed at screening for nutritional deficiencies and searching for signs and symptoms of other autoimmune disorders, gastrointestinal cancers, and refractory sprue. If the patient is doing well without clinical symptoms and has normal antibody titers, he or she should continue to be followed annually. If the patient is doing poorly, indicated by symptoms, nutritional deficiencies, or elevated antibodies, they should again be evaluated by the gastroenterologist with consultation with a knowledgeable dietitian [103]. This patient will also require closer monitoring for the development of the aforementioned nutritional, autoimmune, and possibly malignant complications.

The reader is invited to review separate chapters in this book about additional nutritional considerations in colon cancer, type 1 diabetes, gastroesophageal reflux disease, diarrhea, constipation, lactose intolerance, liver disease, food allergy, osteomalacia, and osteoporosis, all of which can complicate celiac disease.

VI SUMMARY

The astute clinician must have a high index of suspicion to make the diagnosis of celiac disease. Although this condition is very common, it is under diagnosed because of its protean manifestations. The diagnosis of this disease is further complicated by its presentation at any age with a variety of symptoms ranging from joint pain to infertility and anemia. Serum antibodies are an excellent screening tool for this disease. However, confirmation requires a small bowel biopsy performed by a gastroenterologist via an upper endoscopy. Celiac disease is the only autoimmune disease for which we know the trigger: gluten. Removal of gluten from the diet results in complete histological and symptomatic recovery in the majority of patients. Not uncommon complications of celiac disease can include vitamin and mineral deficiencies, the development of other autoimmune diseases, and a higher risk for osteoporosis and gastrointestinal cancers. We now recognize that up to 15% of pediatric patients and nearly 2/3 of adult patients with celiac disease may have persistent autoimmune enteropathy even after 2 years on a gluten-free diet [142,143]. Living gluten-free is challenging socially and the potential for contamination of foods by wheat, rye, and barley is high. The patient benefits best from the involvement of a physician, a dietitian, and a support group who are up-to-date about the diet as well as the latest literature and advances in the understanding of the complex interactions between gluten and the immune system.

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Chapter 42

Nutrition and Cystic Fibrosis

Zhumin Zhang, Lyanne H. Chin and HuiChuan J. Lai
University of Wisconsin, Madison, WI, United States

I OVERVIEW OF CYSTIC FIBROSIS

Cystic fibrosis (CF) is one of the most common, life-shortening autosomal recessive disorders with estimated incidences of approximately 1 in 3000 White live births, 1 in 17,000 Black live births, and 1 in 90,000 Oriental live births [1,2]. CF was recognized as a distinct clinical entity in 1938. It is a generalized disease of the exocrine glands characterized by abnormal sodium and chloride transport, leading to elevated electrolyte levels in sweat [3,4]. Dysfunction of the other exocrine glands occurs producing viscid secretions of low water content. This results in pancreatic insufficiency (PI), which results in malabsorption and failure to gain weight, as well as airway obstruction, which leads to increased susceptibility to recurrent bronchial infection, progressive lung damage, and eventual respiratory failure.

A Clinical Presentation

There are three categories of major clinical abnormalities in CF: (1) gastrointestinal tract involvement, characterized by PI leading to malabsorption and malnutrition; (2) respiratory tract involvement, characterized by recurrent infections and chronic obstructive pulmonary disease; and (3) salt loss in sweat that can lead to severe hyponatremic dehydration. The pancreatic disturbance begins prenatally and can cause intestinal obstruction in newborns with CF, a problem referred to as meconium ileus (MI). It has been estimated that 15−20% of CF patients have MI [5], 85−90% are pancreatic insufficient (PI), and 10−15% are pancreatic sufficient (PS) [6].

More recent studies have shown that PI develops during the first year of life such that about half the patients destined to have this problem show intestinal malabsorption by about 1 month of age, two-thirds by 6 months, and the remainder by 1 year [7]. Unlike PI, pulmonary status of patients with CF often appears normal at birth [8]; however, it inevitably shows obstruction and infection. The onset and rate of progression of CF lung disease are not well understood but appear to vary widely among individuals [8−11]. Other complications may occur as the disease progresses. For example, glucose intolerance and diabetes mellitus may develop [12,13]. The prevalence of CF-related diabetes (CFRD) is reported to be between 5% and 15% in children 6−17 years old and up to 35% in adults with CF [14−18]. Up to 11% of CF patients develop overt liver disease because of focal biliary cirrhosis [14]. Due to an absent vas deferens at birth (presumably resulting from ductal obstruction with dehydrated secretions), infertility in males with CF is virtually universal [19]. About 16% of patients reported in 2013 CF Foundation (CFF) Patient Registry had bone disease [14].

B Pathogenesis

On the basis of molecular genetics research [20], CF fundamentally can be attributed to mutations occurring in the long arm of chromosome 7. With cloning of the CF gene, it has been demonstrated that the most common mutation is a 3 base pair (bp) deletion, this results in the loss of a phenylalanine residue at amino acid position 508 of the predicted gene product, namely the cystic fibrosis transmembrane conductance regulator (CFTR) [21−23]. The 3 bp deletion mutant, commonly referred to as F508del, occurs in about 70% of the CF chromosomes [21] and more than 85% of CF patients in the United States have at least one F508del allele [24]. However, over 2000 other DNA mutations in the CFTR gene have been identified (http://www.genet.sickkids.on.ca/cftr/app) and more are still being found. Most mutations occur infrequently, and few (less than 10%) are common enough to be well characterized (http://www.cftr2.org) [25]. Among these gene

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mutations, some are disease-causing, some are sequence variations that do not cause CF, some are associated with single or milder organ system involvement than typically seen in CF, and some have variable or unknown consequences. The severity of CF is determined by both copies of CFTR mutations [26,27], and further influenced by modifier genes [28,29], environment, and socioeconomic status of patients [30–33].

CFTR is a cAMP-regulated anion channel expressed primarily at the apical plasma membrane of secretory epithelia. The abnormal or absent CFTR protein is the underlying pathogenic factor in CF disease process due to its role in regulating ion transport across the apical membrane of epithelial cells, particularly chloride conductance, which is invariably defective in CF [2,4]. This defect leads to abnormally high chloride concentration in the sweat, which constitutes the classical diagnostic test for CF [34]. Since the discovery of CFTR gene in 1989 [20], research has focused on addressing the underlying genetic defect of CF. The potential strategies, including gene therapy targeting genetic mutations and the search for CFTR modulators targeting the defect protein, offer great promises for life-saving treatments for CF patients. In the past several years, landmark clinical trials have shown that correction of CFTR function leads to substantial clinical benefits for patients with CF [35,36]. Moving forward, basic and translational CF research remains essential to develop effective disease-modifying therapies and to make them available to all patients with CF.

C Diagnosis and Treatment

Individuals with suspected CF are identified for diagnostic evaluation after newborn screening (NBS), recognition of a positive family history, and/or following the development of the characteristic signs and symptoms such as steatorrhea or chronic cough. Diagnosis then may be made through various approaches, depending on age and phenotype, but ultimately the sweat chloride test is the key to diagnosis and should be used according to published diagnostic criteria [37].

CF NBS provides an opportunity for presymptomatic detection on a routine basis before irreversible pathology develops [8]. After benefits, particularly nutritional advantages [38–42], were demonstrated that outweigh the risks, the CDC recommended universal screening for CF [43]. Now, almost all the babies born in the United States and over 8 million newborns are screened annually for CF worldwide. The use of NBS for CF is based on initial detection of elevated immunoreactive trypsinogen (IRT) levels [44,45]. The discovery of the CFTR gene in 1989 [20] has promoted the development of new screening methods where the IRT test is coupled with a multipanel CFTR mutation analysis [46,47]. The standard NBS protocols begin with a first-tier phenotypic test that measures IRT in dried blood spots. Infants who have an elevated IRT are then referred for further testing, either a repeat IRT test at 2 weeks of age or DNA analysis for CFTR mutations. Infants with a second positive screening result are referred to sweat testing to establish the diagnosis of CF. It is important to understand that a positive IRT test alone is not equivalent to a diagnosis of CF. In fact, only about 1% of newborns with positive IRT have CF. In addition, not all CF cases are detected by screening for IRT; approximately 5% of CF patients show false-negative IRT in their dry-blood specimens obtained at birth [48]. There are various NBS protocols being implemented across the world. Variations in the effectiveness of individual programs warrant further quality improvement [49,50].

Without therapy, CF is usually fatal within the first decade of life. Current treatment is multifaceted and requires close monitoring by an expert multidisciplinary care team. This care should include regular evaluation, counseling, and intervention by expert physicians, nurses, dietitians, respiratory and/or physical therapists, and social workers. Genetic counselors, psychologists, and exercise physiologists are also important resources. Clinical management of CF involves treatment programs with three principal objectives: (1) improve nutritional status, (2) promote clearance of respiratory secretions, and (3) control bronchopulmonary infections. Care programs for CF patients in North America and many European countries are organized in specialized regional centers. These centers have placed particular emphasis on enhancing nutrition and using aggressive strategies to prevent progressive pulmonary disease [51]. Although there is currently no cure for CF, treatment programs have been generally effective, as evidenced by the increasing longevity of CF patients in the United States from less than 20 years to 40.6 years over the past three decades [14,52]. The primary causes of death in patients with CF are cardiorespiratory complications, accounting for about 80% of deaths. For this reason, most CF centers in the United States place a great deal of emphasis on respiratory management for patients with CF. In addition, with diagnosis through NBS, an increased emphasis has been placed on preventing malnutrition in the recent decade.

In the new era of nationwide CF NBS, many CFTR-associated abnormalities, in addition to the classic CF, have been identified, for example, CFTR-related disease, CFTR-related metabolic syndrome, CF screen positive, inconclusive diagnosis, and delayed CF [53]. With limited data on long-term outcomes, optimal clinical management for these patients is unclear. Clinical management has been inconsistent and difficult, despite published recommendations and guidelines [54–57], which are largely consensus-based and not evidence-based.

D Consequences of Malnutrition

Evidence has accumulated in recent years from longitudinal studies that the consequences of malnutrition in CF are more severe than previously appreciated from clinic-based cross-sectional observations. Long-term adverse consequences of malnutrition include: (1) permanently stunted growth [38–40]; (2) cognitive dysfunction [58,59]; and (3) greater susceptibility to lung disease [60–63]. More research is underway to address whether early nutritional intervention could alter long-term pulmonary disease progression.

II MALNUTRITION IN CF

CF is associated with an increased risk of protein-calorie malnutrition, as well as deficiencies in fat-soluble vitamins and other micronutrients. Malnutrition associated with CF is characterized by its early onset, and is often present at the time of CF diagnosis. At the mild end of the malnutrition spectrum, CF patients may have depleted stores or low circulating concentrations of a given nutrient, but no associated signs or symptoms. More pronounced nutritional deficiencies lead to metabolic abnormalities, structural changes, functional disturbances, growth failure, developmental delay, and a variety of other characteristics of malnutrition. Malnutrition is most likely to occur during periods of rapid growth when nutritional requirements are high, during pulmonary exacerbations, and with increased severity of lung disease.

Growth impairment, abnormalities in the biochemical markers of nutritional status, and clinical symptoms of malnutrition all have been reported in patients with CF. Historically, malnutrition in patients with CF was thought to represent either an inherent consequence of the disease process or a physiologic adaptation to advanced pulmonary disease. However, it is now recognized that there are multiple causes of malnutrition in CF and they can be attributed to three primary mechanisms [64–66]: increased energy and nutrient losses, increased energy expenditure, and decreased energy and nutrient intakes. Table 42.1 lists the major risk factors for malnutrition associated with CF.

TABLE 42.1 Factors Contributing to Malnutrition in CF

Disease Factors:
- Presence of PI
- Severity of PI (the degree of steatorrhea and azotorrhea)
- Partial intestinal resection secondary to bowel obstruction (caused by MI)
- Severity of respiratory disease
- Loss of bile salts associated with steatorrhea
- Cholestatic liver disease
- Diabetes mellitus

Nutritional Factors:
- Growth rate (of particular concern in young children and adolescents with CF)
- Energy and macronutrient intakes (e.g., the quantity and quality of food consumed)
- Micronutrient deficiencies (e.g., fat-soluble vitamins)
- Energy expenditure
- Eating behaviors

A Causes of Malnutrition

1 Increased Losses

Loss of nutrients from maldigestion and malabsorption as a result of PI is the primary factor contributing to energy and nutrient deficiencies in patients with CF [67]. The ductular cells of the pancreas respond to stimulation with secretin by producing a high-volume, bicarbonate-rich secretion. This secretion functions to neutralize gastric acid, thus enabling the pancreatic digestive enzymes to function at their optimum pH. The abnormal chloride transport caused by the defected CFTR protein leads to thickened secretions that obstruct the pancreatic ducts and prevent the secretion of enzymes and bicarbonate. In CF, PI is defined by the presence of measurable steatorrhea. This does not occur until 1–2% of pancreatic enzymatic capacity remains [68]. Therefore, CF patients with PI have severe, irreversible loss of pancreatic function. It is also important to understand that CF patients with pancreatic sufficiency (PS) do not have normal pancreatic function [69]. They have decreased volume of bicarbonate-rich secretion but continue to produce enough pancreatic enzymes to avoid steatorrhea.

Maldigestion and malabsorption caused by PI can be attributed to three major abnormalities: lack of digestive enzymes, inadequate bicarbonate secretion, and loss of bile salts and bile acids. Inadequate bicarbonate secretion results in impaired capacity to neutralize gastric acid in the duodenum, and a lower intestinal pH until well into the jejunum, which often reduces the effectiveness of pancreatic enzyme replacement therapy (PERT) [67–69] (see Section IV.A.2, Pancreatic Enzyme Replacement Therapy). Loss of bile salts and bile acids often exacerbate maldigestion and malabsorption. Bile acids are readily precipitated in an acidic milieu, and duodenal bile acid concentration may fall below the critical micellar concentration, thereby exacerbating fat maldigestion. Precipitated bile salts also appear to be lost from the enterohepatic circulation in greater quantities, thus reducing the total bile acid pool and altering the glycocholate: taurocholate ratio. Oral taurine supplements have been reported to benefit some CF patients [70].

Other factors also contribute to energy and nutrient losses in CF. Patients presenting with MI, in particular

those who have undergone intestinal resection, have further reduction in intestinal absorptive capabilities. Viscid, thick intestinal mucus, with altered physical properties, may affect the thickness of the intestinal unstirred layer, further limiting nutrient absorption. CFRD may increase caloric losses due to glycosuria if not adequately controlled. Advanced liver disease and biliary cirrhosis may result in reduced bile salt synthesis and secretion, which may lead to severe fat malabsorption.

2 Increased Requirement

Energy requirements in patients with CF are highly variable. Several studies have reported that patients with CF have an associated increased energy expenditure compared with non-CF patients [71–74]. A variety of explanations have been proposed to explain the increased energy expenditure observed in CF patients. These include chronic respiratory infection, increase in work of breathing, genetic and cellular defects, and changes in body composition.

Chronic respiratory infections, particularly with *Pseudomonas aeruginosa*, have been shown to be associated with 25–80% increase in metabolic rate and energy requirements [75]. The link between CF genotype and energy requirement was reported in a study by Tomezsco et al., who demonstrated that energy expenditure was increased by 23% in CF patients with homozygous F508del mutations as compared with non-CF controls [74]. Therefore, a patient with advanced lung disease might not be able to ingest sufficient calories to meet energy needs.

The hypothesis that a basic cellular defect may increase energy requirement was supported by in vitro studies showing that mitochondria from cultured fibroblasts obtained from CF patients had higher rates of oxygen consumption compared with control tissues [76,77]. When F508del was identified, it was proposed that the defective CFTR protein may affect cellular energy metabolism through its involvement in the regulation of ion transport across membranes since CFTR is a cAMP-regulated chloride channel [4].

3 Decreased Consumption

The appetite or caloric intake of CF patients may be limited due to a variety of disease complications. Acute pulmonary exacerbations are a common cause of anorexia, and respiratory infections often give rise to nausea and vomiting, which may further reduce caloric intake [63]. The biochemical causes of anorexia associated with acute infection are unclear, but elevated circulating levels of tumor necrosis factor may play a role [78].

In addition to pulmonary complications, a variety of gastrointestinal complications also contribute to anorexia and inadequate caloric intake [64]. Increased occurrence of gastroesophageal reflux disease (GERD) and esophagitis are observed in patients with CF. Distal intestinal obstruction syndrome (DIOS), a form of subacute or chronic partial bowel obstruction, usually occurs in older patients with PI. Large fecal masses palpable in the abdomen give rise to intermittent abdominal distention and cramping accompanied with reduced appetite. Constipation in the absence of DIOS is another cause of anorexia and abdominal discomfort in older patients with CF.

A number of dietary surveys indicate that CF patients often eat less than normal, particular during the period of 1970s and early 1980s, when CF patients were commonly prescribed with fat-restricted diets on the assumption that a reduction in dietary fat intake might improve bowel symptoms [79]. The observation of better growth and survival in CF patients who received unrestricted-fat, high-calorie diet in combination with PERT compared with those who received low-fat diet in the early 1980s has changed dietary practices in most CF centers [80,81]. Energy intakes of 110% or greater than the estimated energy requirement (EER) with 35–40% of energy from fat are now recommended for patients with CF [82,83]. However, CF patients often fail to consume such high quantities of calories and/or fat because of their disease manifestation. In several cross-sectional or short-term studies [84–88] and a small prospective 3-year study of 25 patients [89], energy and fat intakes of CF patients were reported to be much lower than these recommendations. A longitudinal study evaluating dietary intake patterns in children with CF from the time of diagnosis to age 10 years revealed mean energy intake was at ~110% of EER with fat consisting of ~37% of energy [39].

B Common Nutritional Deficiencies

1 Energy and Macronutrients

As discussed earlier, patients with CF are at high risk of energy deficiency due to their increased requirement and decreased consumption. Protein poses less of a nutritional problem than fat in the CF population. The major risk of protein deficiency in CF patients occurs during the first year of life, when the average requirement is at least three times as great as that in adulthood. Low serum markers of protein (e.g., albumin, prealbumin, and retinol binding protein) are commonly found in infants and young children with newly diagnosed CF. One-third to one-half of infants diagnosed through CF NBS were reported to be hypoalbuminemic [90–92]. Normalization of serum albumin level often occurs following comprehensive nutrition therapy [93].

The consequence of energy deficiency leads to impaired growth in children with CF. Weight retardation and linear growth failure are the most common observations documented in CF clinics [94,95], although its severity and prevalence vary greatly. Accurate estimates on the prevalence of malnutrition in the CF population

have been difficult to obtain in the past, due to lack of sufficient data. In recent years, comprehensive national databases, known as CF Patient Registries, have been compiled by the U.S. and Canadian CFFs, as well as the European CF Society, making it possible to determine population estimates on the prevalence of malnutrition associated with CF. Analysis on 13,000 pediatric CF patients documented in the 1993 U.S. CF Patient Registry revealed that the growth of CF children at all ages were mostly below normal [96]. Malnutrition was particularly prevalent in infants (47%) and adolescents (34%) as compared with children at other ages (22%), and in patients with newly diagnosed, untreated CF (44%). Underweight is also prevalent in adults with CF; approximately 35% of the 7200 adults with CF documented in the 1992–94 U.S. and Canadian CF Patient Registries were found to be underweight [97]. During the past decade, the prevalence of malnutrition has decreased steadily from ~25% in 1995 to ~15% in 2005 in pediatric CF patients [98].

2 Essential Fatty Acids

Essential fatty acid deficiency (EFAD) has been known to occur in CF patients [90,97,99,100]. During infancy, particularly before diagnosis, EFAD can occur with desquamating skin lesions, increased susceptibility to infection, poor wound healing, thrombocytopenia, and growth retardation. In patients who are adequately treated, clinical evidence of EFAD is rare, although biochemical abnormalities of essential fatty acid (EFA) status remain common [101–103]. Abnormal unsaturated fatty acids are more common in patients with PI than those with PS [104]. The major fatty acid abnormalities found in patients with CF are low levels of linoleic (18:2, *n*-6) and docosahexaenoic acids (22:6, *n*-3); alterations in arachidonic acid (20:4, *n*-6) level; and elevated levels of palmitoleic (16:1, *n*-7), oleic (18:1, *n*-9), and eicosatrienoic (20:3, *n*-9) acids. In EFAD, oleic acid is converted to eicosatrienoic acid, which is commonly referred to as the "pathologic triene" because its increase in EFAD, coupled with a decrease in arachidonic acid, leads to a high triene/tetraene ratio [93,105,106]. Multiple hypotheses have been proposed to explain the underlying mechanisms of abnormal EFA status associated with CF. Fat malabsorption secondary to PI is the most common explanation for EFA abnormalities. However, some investigators have postulated a primary metabolic defect in fatty acid metabolism [101–108].

Many studies have reported a clear association between better EFA status and better growth in children with CF. Plasma linoleic acid was shown to be correlated positively with growth in children whose CF was diagnosed before 3 months and followed up to 12 years of age [90]. Another study by van Egmond et al. showed better growth among CF infants who consumed a predigested formula that contained high linoleic acid (12% of energy) compared with those who consumed a comparable formula with lower linoleic acid (7% of energy), despite a lower total energy intake in the former group [109]. More recently, Shoff et al. demonstrated that, in children who experienced longer and more severe malnutrition due to delayed diagnosis, maintaining normal plasma linoleic acid (i.e., > 26% of total plasma fatty acids) in addition to sustaining a high-caloric intake (i.e., >120% of EER) is a critical determinant in promoting catch-up weight gain [110].

Despite the above evidence, EFA supplementation for CF patients remains controversial for several reasons. Firstly, not all patients respond to EFA supplementation; normalizing plasma linoleic acid is particularly difficult in patients with MI [66,74]. Secondly, *n*-6 fatty acids, including linoleic acid and its metabolite arachidonic acid, have been proposed to play a role in CF inflammation [111–115]. In the 2009 CFF infant care guidelines [116], clinical trials to "answer questions related to EFA supplementation in infants" are recommended as a priority of research to resolve this long, overdue controversy. Since then, a pilot study examining the effects of *n*-3 supplementation in children and adults showed that EPA and DHA supplementation for 3 months exhibited slow yet important clinical benefits regarding exacerbations and use of antibiotics. However, larger studies are required to ascertain the conclusions [117]. A recent review summarizing findings from four small studies also demonstrated insufficient evidence of the benefits of *n*-3 fatty acids supplementation [118].

3 Fat-Soluble Vitamins

Deficiencies of fat-soluble vitamins in the CF population have been demonstrated in many studies [89,91,114,115,119–126]. Vitamins A and E are of the greatest concern, particularly in patients with severe malabsorption or liver disease. However, recent studies show that deficiencies in vitamin D or vitamin K are also common, especially in CF patients with advanced cholestatic liver disease. Abnormalities in fat-soluble vitamins are particularly prevalent in newly diagnosed infants with CF. Studies on infants diagnosed through NBS showed that 20–40% had low serum retinol, 35% had low serum 25-hydoxyvitamin D, and 40–70% had low serum α-tocopherol [90,91,119].

Vitamin A deficiency was the first micronutrient deficit demonstrated in patients with CF. Clinical symptoms of vitamin A deficiency reported in CF patients include keratinizing metaplasia of the bronchial epithelium, xerophthalmia, and night blindness. Pancreatic lipase is required to digest retinyl esters prior to absorption. Other mechanisms for vitamin A deficiency in CF has been proposed, ranging from a defect in the mobilizing hepatic storage of vitamin A due to liver disease to low levels of retinol binding protein, which is responsible for transporting vitamin A in the

circulation [119,120]. More recently, elevated vitamin A intake and serum retinol have been of growing concern, especially among preadolescents and young adults. Excessive vitamin A intake may increase risks of bone complications. Although supplementation can prevent vitamin A deficiency in CF, caution should be exercised to ensure adequacy and avoid toxicity [121,122].

Vitamin E deficiency in CF is most commonly evidenced by low plasma levels of α-tocopherol and has been associated with hemolytic anemia [123]. Low α-tocopherol levels are prevalent in infants with CF identified with NBS programs [90,119]. Those with early, prolonged severe deficiency may also show cognitive dysfunction [58].

Recent evidence has also suggested vitamin D deficiency is prevalent in both children and adults with CF [125–127]. Suboptimal vitamin D status has been directly linked to poor bone mineralization in CF patients, although the cause of CF bone disease is multifactorial and not completely understood [125]. In the recent years, CFF developed evidence-based, age-specific, and stepwise approach recommendations for achieving and maintaining optimal vitamin D status [128].

Vitamin K deficiency has not been routinely demonstrated in patients with CF. However, vitamin K deficiency is likely to develop in CF patients with severe cholestatic liver disease, short-bowel syndrome, and lung disease requiring frequent antibiotic use [129–131]. Low levels of vitamin K are also seen in patients with CF not taking appropriate vitamin supplementation. Vitamin K status can be evaluated by prothrombin time or the more sensitive protein induced by vitamin K absence (PIVKA) measurement.

4 Minerals and Electrolytes

For patients with CF, sodium is of great concern because of its abnormally high content in the sweat. Therefore, sodium requirement may be considerably higher for CF patients than normal individuals. Salt depletion can be catastrophic, leading to severe hyponatremic dehydration and shock [132,133]. In addition, there has been concern that marginal or low body sodium may limit the growth of children with CF [134]. Young infants with CF are particularly at risk for hyponatremia, hypochloremic dehydration with metabolic alkalosis, irritability, and lethargy. Therefore, electrolyte status should be evaluated if there is any suspicion of electrolyte depletion, such as exposure to high environmental temperatures, over-bundling or excessive sweating. Serum sodium levels do not accurately reflect total body sodium; urinary sodium in relation to creatinine is a better indicator of sodium status in infants. Because of increased risk of hyponatremia, infants with CF should receive salt supplementation. Current guidelines recommend a daily dose of one-eighth teaspoon of table salt, which contains 12.5 mEq of sodium, for infants younger than 6 months; for infants age 6–12 months, a quarter teaspoon per day, but not to exceed 4 mEq/kg/day, was recommended [116]. In older children and adults with CF, although routine sodium supplements may not be necessary because the average American diet contains an overabundance of sodium, sodium supplements are definitely needed in conditions that may cause prolonged sweat loss.

Low calcium intake and suboptimal bone density accrual are of concerns even in the general pediatric population [135]. Children with CF have a higher risk of bone diseases because of poor growth and delayed puberty, malabsorption of nutrients needed for bone health (e.g., calcium, vitamins D and K), reduced physical activity, pulmonary inflammation with increased cytokines, and medication such as glucocorticoid therapy [125]. The relative contribution of these factors to development of bone disease in patients with CF remains to be elucidated. Little data are available regarding calcium supplementation in improving bone health in the CF population. A recent double-blind, cross-over, randomized trial with 15 CF children aged 7–13 years showed that supplementation with calcium (1000 mg), vitamin D_3 (2000 IU) or both for 6 months did not change serum calcium and 25-hydroxy-vitamin D concentrations and bone mineral gain, compared to the placebo (400 IU of vitamin D_3) group [136].

Stable isotope studies have reported increased fecal zinc losses and decreased zinc absorption in infants and children with CF [137,138]. Zinc deficiency affects growth and vitamin A status but is difficult to identify because serum zinc is not an adequate measure for zinc status. Therefore, current CFF guidelines recommend a trial of zinc supplementation, 1 mg/kg/day elemental zinc for 6 months, to CF children experiencing poor growth despite adequate caloric intake and pancreatic enzyme supplementation [82,116].

Anemia in CF has been reported with varying prevalence as high as 33%, with iron deficiency proposed to be the main cause [139]. Chronic lung inflammation may also alter iron metabolism. CF patients with advanced pulmonary disease have been shown to have low serum ferritin levels [139,140]. In the general pediatric population, the importance of diagnosis and prevention of iron deficiency has been emphasized recently, stemming from new evidence showing the adverse, long-term and irreversible effect on neurodevelopment and behavior caused by iron deficiency [141]. In children with CF, chronic lung inflammation poses additional risk for iron deficiency and anemia. In patients with CF, serum transferrin receptor is a more sensitive indicator of iron status than serum ferritin, because the latter is an acute phase reactant to inflammation, which may be artificially elevated with the presence of lung disease.

III NUTRITION ASSESSMENT

Frequent monitoring of the nutritional status for patients with CF is essential to ensure early detection of any deterioration and prompt initiation of nutrition intervention. Patients with CF are most vulnerable to experience malnutrition due to delayed diagnosis, during times of rapid growth (e.g., infancy and adolescence), and during pulmonary exacerbations. During these periods, close monitoring and intervention are critical to prevent nutritional decline. It should be emphasized that with comprehensive nutrition assessment and intervention, children with CF who are diagnosed early through NBS can achieve normal growth throughout childhood [38,39], and adults with CF can maintain normal weight status. In addition, optimizing growth and nutritional status is critical for CF patients, because malnutrition worsens lung disease, affects the quality of life, and reduces survival [94,142].

Assessment of nutritional status for patients with CF must include anthropometric, biochemical, clinical, and dietary assessments. The frequency at which the different indices of nutritional status monitoring should be measured is given in Table 42.2.

A Anthropometric Assessment

Anthropometric assessment, with an emphasis on physical growth in children and body weight in adults, is an important component of nutritional assessment in patients with CF. For children with CF, accurate and sequential measurements of head circumference (age 0−3 years), recumbent length (age 0−2 years), height (age 2−20 years), and weight (age 0−20 years) should be obtained at each clinic visit using standardized techniques. For adults with CF, body weight should be measured at each clinic visit. Other anthropometric assessments, such as skinfold measures (e.g., midarm circumference, triceps skinfold, etc.) provide additional information on body composition, that is, lean body mass and subcutaneous fat stores. However, skinfold measures are prone to measurement errors and reference standards are not available for all ages of children.

Growth measurements should be plotted on appropriate growth charts and converted to sex- and age-specific percentiles. For children with CF younger than 24 months, 2006 World Health Organization growth reference is recommended as a growth standard [143,144] to replace 2000 CDC growth reference, which is recommended for older children at age 24 months or above. Comparisons between the use of the CDC and WHO growth charts in CF revealed significant differences [145]. Concerns and confusions arise when switching growth references, as the transition may create disjunction by changing how a child's growth is assessed if the same cutoff values for malnutrition were applied. The current nutritional goal and malnutrition criteria in children younger than 24 months are set based on CDC growth charts, which need to be revised for the WHO charts.

Nutrition goals for weight and stature in patients with CF are summarized in Table 42.3. Patients with CF should aim at maintaining body mass index (BMI), ≥50th percentile for children age 2 years and older, ≥22 for female adults, and ≥23 for male adults, to support better lung function [82]. For children younger than 24 months, weight-for-length percentile at 50th percentile or above is recommended. However, this cutoff value is set for the CDC growth charts, which needs to be modified for the use of the WHO charts. With regard to linear growth, in addition to determining height-for-age percentile, it is important to evaluate whether the child's height is reaching his/her genetic potential. This can be estimated by using parental heights to calculate a target height range or an adjusted height percentile [82,146,147]. CF children whose heights are below their genetic potential are considered at risk and should be evaluated [82,83]. However, the target height method to estimate genetic potential suggested by CFF has flaws [147], and should be used with caution. In addition to weight, weight-for-stature, and length/height percentiles, weight gain and height velocity are more sensitive indicators of growth, and should be evaluated, especially when growth faltering is observed [82,116,148]. For young children with CF, appropriate weight gain needs to be maintained at or above 50th percentile expected for age.

B Biochemical Assessment

Monitoring biochemical indices of nutritional status is essential in patients with CF [82]. Current guidelines (Table 42.2) recommend routine, annual measurements of serum protein (albumin), vitamin A (retinol), vitamin D (25-hydroxycholecalciferol), vitamin E (α-tocopherol), and iron (hemoglobin, hematocrit).

Assessment of EFA status is not routinely performed but only as indicated. However, recent findings on the relationships between abnormal EFA status and growth in children with CF [90,91,110], particularly those with MI, warrant the consideration of routine monitoring of EFA status, at least annually, in patients with CF. Similarly, routine measurements of vitamin K, calcium, zinc, and sodium are not regarded as necessary in current guidelines [82,116], but may be needed in individual patients.

C Clinical Assessment

Clinical assessment of nutritional status in children with CF focuses on evaluation of the severity of maldigestion and malabsorption caused by PI. A total of 85−90% of

TABLE 42.2 Nutritional Assessment, Monitoring in CF

	At Diagnosis	Early Infancy (0–6 Months)					Late Infancy (7–12 Months)			Second Year		Age 2–5 Years		Age 6–20 Years		Age 20 + Years	
Age at visit	1 month	2 months	3 months	4 months	5 months	6 months	8 months	10 months	12 months	Every 2–3 months	24 months	Every 3 months[a]	Annual	Every 3 months	Annual	Every 3 months	Annual
Anthropometric assessment																	
Weight, stature, weight gain[b]	x	x	x	x	x	x	x	x	x	x	x	x		x		x	
Head circumference (up to age 3 years)	x	x	x	x	x	x	x	x	x	x	x	x		x			
BMI (ages > 2 years)									x			x		x		x	
Skinfold measures													x		x		
Pubertal status (Tanner stages)															x[c]		
Biochemical assessment																	
Complete blood count		x					x		x		x						
Albumin		x					x		x		x		x				
Essential fatty acids[d]		x					x		x								
Vitamins A, D, and E, iron[e]		x					x		x		x		x				
Vitamin K[f] and zinc[g]																	
Calcium and bone status[h]																	
Serum electrolytes,[i] creatinine, glucose											x						

(*Continued*)

TABLE 42.2 (Continued)

	At Diagnosis	Early Infancy (0–6 Months)					Late Infancy (7–12 Months)			Second Year		Age 2–5 Years		Age 6–20 Years		Age 20 + Years	
Age at visit	1 month	2 months	3 months	4 months	5 months	6 months	8 months	10 months	12 months	Every 2–3 months	24 months	Every 3 months[a]	Annual	Every 3 months	Annual	Every 3 months	Annual
Clinical assessment																	
Pancreatic functional[j]	X								x		x		x				
Dietary assessment																	
Energy and nutrient intake[k]	X	x	x	x	x	x	x	x	x	x	x	x		x			
PERT dosing	X	x	x	x	x	x	x	x	x	x	x	x		x			
Feeding/eating behavior				x				x			x			x			

[a]*For children ages 2–5 years at nutritional risk, the 2016 preschoolers guidelines patients be seen in 8 weeks or sooner [183].*
[b]*For children ages 0–5 years, the infants and preschoolers guidelines recommended desired age-specific weight gain [116,183].*
[c]*Starting at age 9 years for girls and 10 years for boys until sexual maturation completes, annual pubertal self-assessment (patients, or parent and patient) or physician assessment using Tanner stage system [175,176]; annual question as to menarchal status for girls.*
[d]*The 2002 guidelines recommended checking EFA status in children with failure to thrive [82]. Based on abundant literature (see text) and recent new findings [110], we recommend routine monitoring of EFA status in young infants, age 1 year and 2 years.*
[e]*Consider checking serum transferrin receptor levels for iron status.*
[f]*In patients with liver disease or if patient has hemoptysis or hematemesis [82]; recommended tests include PIVKA-II (preferred) or prothrombin time.*
[g]*In children with poor growth despite adequate caloric intake and PERT [82,116]; no recommended test as serum zinc does not reflect zinc sufficiency, instead, a trial of zinc supplementation should be given (see text).*
[h]*In patients > 8 years of age if risk factors are present (see text); recommended tests include serum calcium, phosphorus, ionized parathyroid hormone, and dual-energy x-ray absorptiometry.*
[i]*If patients exposed to heat stress and become dehydrated; recommended tests include serum sodium and spot urine sodium [82,183].*
[j]*Recheck a measure of pancreatic function if PS patient have weight loss or gastrointestinal symptoms.*
[k]*A review of enzymes, vitamins, minerals, oral, or enteral formulas, herbal, botanical, and other complementary and alternative medicine.*
Sources: Adapted from D. Borowitz, R.D. Baker, V. Stallings, Consensus report on nutrition for pediatric patients with cystic fibrosis, J. Pediatr. Gastroenterol. Nutr. 35 (2002) 246–259; D. Borowitz, K.A. Robinson, M. Rosenfeld, S.D. Davis, K.A. Sabadosa, S.L. Spear, et al., Cystic fibrosis foundation evidence-based guidelines for management of infants with cystic fibrosis, J. Pediatr. 155 (2009) S73–S93; T. Lahiri, S.E. Hampstead, C. Brady, C.L. Gannon, M.E. Condren, M.F. Guill, et al., Clinical practice guidelines from the Cystic Fibrosis Foundation for preschoolers with cystic fibrosis, Pediatrics 137 (2016) 1–26; J.R. Yankaskas, B.C. Marshall, B. Sufian, R.H. Simon, D. Rodman, Cystic fibrosis adult care: consensus conference report, Chest 125 (2004) 1S–39S.

TABLE 42.3 Nutrition Goals for Weight and Height/Length Status in Patients with CF

Children with CF			Adults with CF
<2 years	2–5 years	6–18 years	>18 years
Normal weight and length status (similar to non-CF infants)	Weight-for-age ≥10th percentile		
Weight-for-length ≥50th percentile	BMI ≥50th percentile	BMI ≥50th percentile	BMI ≥23 (males)
			BMI ≥22 (females)
Stature-for-age percentile at genetic potential (all patients with CF)			
Weight gain >50th percentile expected for age (≤6 years)			

Sources: Adapted from the U.S. and European consensus reports: D. Borowitz, R.D. Baker, V. Stallings, Consensus report on nutrition for pediatric patients with cystic fibrosis, J. Pediatr. Gastroenterol. Nutr. 35 (2002) 246–259; V.A. Stallings, L.J. Stark, K.A. Robinson, A.P. Feranchak, H. Quinton, Clinical Practice Guidelines on Growth and Nutrition Subcommittee. Evidence-based practice recommendations for nutrition-related management of children and adults with cystic fibrosis and pancreatic insufficiency: results of a systematic review, J. Am. Diet. Assoc. 108 (2008) 832–839; A.R. Smyth, S.C. Bell, S. Bojcin, M. Bryon, A. Duff, P. Flume, et al., European Cystic Fibrosis Society standards of care: best practice guidelines, J. Cyst. Fibros. 13 (2014) S23–S42; T. Lahiri, S.E. Hampstead, C. Brady, C.L. Gannon, M.E. Condren, M.F. Guill, et al., Clinical practice guidelines from the Cystic Fibrosis Foundation for preschoolers with cystic fibrosis, Pediatrics 137 (2016) 1–26; J.R. Yankaskas, B.C. Marshall, B. Sufian, R.H. Simon, D. Rodman, Cystic fibrosis adult care: consensus conference report, Chest 125 (2004) 1S–39S; M. Sinaasappel, M. Stern, J. Littlewood, S. Wolfe, G. Steinkamp, H.G. Heijerman, et al., Nutrition in patients with cystic fibrosis: a European Consensus, J. Cyst. Fibros. 1 (2002) 51–75.

CF patients has PI. Pancreatic functional status not only has a direct influence on nutritional status but also is a strong predictor of long-term outcome [69,149]. Data from the 1990–95 U.S. CF Patient Registry demonstrated that patients with PS have an approximately 20-year longer lifespan than PI patients [69].

The clinical signs and symptoms of PI include abdominal discomfort (bloating, flatus, pain), steatorrhea (frequent, malodorous, greasy stools), and the presence of MI or DIOS. Objective tests for PI include: (1) duodenal measurement of pancreatic enzymes and bicarbonate; (2) 72-hour fecal fat balance study; and (3) fecal elastase-1 in spot stool samples. Among these, 72-hour fecal fat balance study has been the gold standard historically. A high-fat diet is ingested for 72 hours, and stool is collected and analyzed for fat excreted. For the most precise results, oral dye markers are used to indicate the period of high-fat ingestion, and the stool that follows the first marker, up to and including the second marker, represents the stool produced during the period of high-fat intake. In clinical practice, diet is often measured for 3 days and stool collected simultaneously. A coefficient of fat absorption, that is (fat intake − fecal fat loss)/fat intake × 100%, is calculated. In CF, PI is defined by a coefficient of fat absorption less than 93% [82].

Since the 72-hour fecal fat balance study is cumbersome and not well accepted by CF patients and care providers, measurement of fecal elastase-1 in a small stool sample has gained wide acceptance and is becoming the standard method of care in most CF centers [69]. Elastase is one of the 20+ enzymes secreted by the pancreas. It has the physical property of being stable as it transits the intestinal tract, unlike other enzymes that may be degraded by intraluminal proteases. As water is withdrawn from the intestinal contents in the colon, elastase concentrations increase, making it easy to measure in stool. This protein is stable through a wide range of pH and temperature, making it ideal to collect at home. Levels greater than 100–200 μg fecal elastase-1 per gram of stool generally indicate PS [69,150].

It is important to assess pancreatic functional status as soon as CF diagnosis is made. Approximately half of CF patients whose initial tests indicate PS become PI later [4]. Therefore, PS patients should be reevaluated at least annually to determine if they have changed to PI, especially if genotype studies reveal mutations that are generally associated with PI. For patients diagnosed with PI, PERT and vitamin supplementation should be started. It is important to understand that although PI can be treated with PERT, it cannot be completely corrected; many patients continue to have steatorrhea when they receive PERT [151]. In addition, response to PERT varies greatly among individual patients.

D Dietary Assessment

Assessments of energy requirement and dietary intakes are important ways of determining whether the patient is at negative energy balance. Evaluation of dietary intake is best performed by dietitians/nutritionists specializing in the care of patients with CF. For patients with good nutritional status, a 24-hour dietary recall may be used to assess dietary habits and the quality of dietary intake. However, for patients with suboptimal nutritional status, a

TABLE 42.4 Method for Estimating Energy Requirement for CF Patients

Step I: Estimate Basal Metabolic Rate (BMR) by Using the WHO Equations		
	Males	**Females**
0–3 years	60.9 × wt − 54	61.0 × wt − 51
3–10 years	22.7 × wt + 495	22.5 × wt + 499
10–18 years	17.5 × wt + 651	12.2 × wt + 476
18–30 years	15.3 × wt + 679	14.7 × wt + 496
>30 years	11.6 × wt + 879	8.7 × wt + 829
Step II: Estimate Energy Expenditure (EE) Using the Following Equation:		
	EE = BMR × (activity coefficient + disease coefficient)	
	Where activity coefficient = 1.3 (confined to bed)	
	1.5 (sedentary)	
	1.7 (active)	
	disease coefficient = 0 (normal lung function, i.e., $FEV_1 > 80\%$)	
	0.2 (moderate lung disease, i.e., FEV_1 40–79%)	
	0.3 (severe lung disease, i.e., $FEV_1 < 40\%$)	
Step III: Estimate Total Energy Requirement (ER), Taking into Account Pancreatic Functional Status		
	a. For PS patients, that is, coefficient of fat absorption (CFA) ≥ 93% ER = EE	
	b. For PI patients with a CFA < 93% ER = EE × (0.93/CFA)	
	c. For PI patients whose CFA has not been determined, use 0.85 as an approximate for CFA ER = EE × (0.93/0.85)	

Sources: D. Borowitz, R.D. Baker, V. Stallings, Consensus report on nutrition for pediatric patients with cystic fibrosis, J. Pediatr. Gastroenterol. Nutr. 35 (2002) 246–259; World Health Organization, Energy and protein requirements, WHO Tech. Rep. Ser. No. 724 (1985) 924.

three-day prospective food record is the best way to obtain quantitative estimates of energy and nutrient intakes. This assessment can then be used as the basis for initiating appropriate nutrition intervention.

Assessment of energy requirement for patients with CF is best determined by estimating the basal metabolic rate, the degree of malabsorption, and the severity of pulmonary disease. For patients older than 2 years of age, current CFF guidelines [83] recommend energy intakes at 110–200% of EER for the general population [145,146] to support weight maintenance in adults and weight gain at an age-appropriate rate in children. Alternatively, the 2002 CFF guidelines [82] provide a method to calculate energy requirement for individual patients based on their pancreatic functional status and the severity of lung disease, as outlined in Table 42.4.

IV NUTRITION MANAGEMENT

Nutrition management for patients with CF varies and depends on the stage of diagnosis (newly diagnosed CF vs routine management), patient's age (infancy, early childhood, adolescence, and adulthood), and disease severity. Nutrition management begins at the time of CF diagnosis. The first 6 months after the diagnosis of CF is a crucial period for establishing therapeutic interventions, dietary counseling, and nutritional education. Nutrition management for patients with stable CF focuses on maintaining optimal nutritional status and preventing malnutrition, and for patients experiencing malnutrition focuses on achieving catch-up growth (for children) and weight gain (for adults). In addition, PERT and vitamin supplementation are essential for all categories of nutrition management.

The multidisciplinary CF care team should monitor growth, provide anticipatory counseling, and plan intervention strategies for each individual CF patient. Achieving and maintaining normal growth and nutritional status require management of gastrointestinal and pulmonary symptoms, dietary intake and eating behaviors, and psychosocial and financial issues. Clinical care guidelines for nutritional management for patients with CF have been established by the U.S. CFF Foundation since 1992 [152], and were revised in 2002 [82] and 2008 [83] to incorporate more evidence-based recommendations. Guidelines for diagnosis care, gastrointestinal care, respiratory care, infection prevention and control care, other CF-related conditions care, as well as age-specific care are also available at https://www.cff.org. The European Cystic Fibrosis Society also updated its standard care guidelines in 2014 [148].

A Diagnosis and Treatment of Malabsorption

1 Diagnosis of PI

Exocrine pancreatic function should be assessed in the following situations: (1) at or shortly after diagnosis to provide objective evaluation of pancreatic status before enzyme therapy is initiated, and (2) to monitor PS patients for evidence of developing fat maldigestion, particularly

when frequent bulky bowel movements or unexplained weight loss occur. The preferred test for assessment of pancreatic functional status is fecal elastase-1, as aforementioned. However, fecal elastase-1 level is not diagnostic by itself but aids in defining PS ($>200\,\mu g/g$) or PI ($<100\,\mu g/g$). The level within the same patient may fluctuate during the first year of life [153]. Therefore, fecal elastase-1 should be remeasured at age 1 year to ensure the correct diagnosis.

2 Pancreatic Enzyme Replacement Therapy

There is a strong association between genotype and pancreatic phenotype [26,27,154]. PERT should be started if the patient is known to have two CFTR mutations associated with PI or objective evidence of PI [116]. PERT should not be started in infants with a CFTR mutation known to be associated with PS, unless there are unequivocal signs or symptoms of malabsorption [116].

In young infants diagnosed through NBS, PI is not present in some infants at the time of diagnosis but develops later in infancy or even in early childhood [7]. Therefore, it is important to repeat fecal elastase-1 measurement in infants who are initially PS, especially when gastrointestinal symptoms appear or poor weight gain occurs. CF children with laboratory evidence of PI should be started on PERT even in the absence of signs or symptoms of fat malabsorption.

Pancreatic enzymes are extracts of porcine origin containing amylase, proteases, and lipase. A large variety of enzyme products have been available previously. However, many were marketed without formal testing. In 2004, the FDA issued a notice requiring that manufacturers submit a new drug application for pancreatic enzyme products. As of 2010, there are three FDA-approved products. The actual activity of these enzymes varies considerably according to specific batches and the commercial manufacturer. Enzyme potency is based on the content of amylase, protease, and lipase in each capsule. However, many health care providers use lipase content to determine enzyme dosing to treat fat maldigestion. Commercial products are sold in capsules with varying lipase activity, ranging from 3000 to 36,000 lipase units/capsule.

The enteric-coated forms of pancreatic enzymes vary considerably in their biochemical coating, biophysical dissolution properties, and size of microspheres or microtablets [155,156]. There are few carefully performed clinical studies comparing the different formulations and little in vivo data are available that demonstrate the superiority of a single product. In fact, all currently available enzyme products fail to completely correct nutrient maldigestion in all patients with CF [157]. The reasons are multiple, and likely to vary from patient to patient, and in some cases may be due to factors unrelated to failed pancreatic digestion [158]. The enteric coating of enzyme microsphere or microtablets require a pH $>5.2-6.0$ for dissolution to occur in the proximal intestine, which may be acidic in the CF patient. Patients with CF and PI have gastric acid hypersecretion and a relative deficiency of bicarbonate secretion from the pancreaticobiliary tree. This may result in a more acidic proximal intestinal environment, which may be below the ideal optimal pH for maximal pancreatic enzyme activity and may hasten the inactivation of enzymes especially lipase within the small intestine. Histamine antagonists or proton pump inhibitors may be used to improve the intestinal milieu, but studies have revealed mixed results [159,160]. Even if nutrient digestion is achieved, malabsorption of nutrients may occur because of thick intestinal mucus, which may affect the unstirred water layer reducing absorption of fatty acids in the small intestinal epithelium [161]. Nevertheless, enzymes do improve nutrient digestion and absorption in CF patients, but the caregiver must be aware of the less than ideal efficacy of these products in individual patients.

a Dosing Guidelines

To date, no studies have been performed in infants to determine the optimal dose of PERT. Data are insufficient with regard to the association of enzyme dose to macronutrient content, coefficient of fat absorption, or growth [83]. Until reliable data are available, dosing is based on consensus recommendations established by the U.S. CFF and the FDA [82,83,162]. These include: 500−2500 units lipase per kg body weight per meal; or $<10{,}000$ units lipase per kg body weight per day; or <4000 units lipase per gram dietary fat per day. These guidelines were established when it was recognized that many CF centers were giving excessive doses of enzymes, which is strongly associated with a severe intestinal complication termed fibrosing colonopathy [162,163].

Response to PERT by individual patients will vary considerably, as will their required dosing schedule. Though dosing is best calculated using lipase units per gram fat ingested, it is perhaps more practical to use a dosing schedule with weight-adjusted guidelines [82,83,116]. Weight-adjusted guidelines, with a limit of 4000 units lipase/g fat or 2500 units lipase/kg/meal beyond 1 year of age, would avoid overdosing.

b Enzyme Administration

There are no convincing data concerning timing of enzyme dosing with meals, but for practical reasons, we recommend that enzymes be taken in 2−3 divided doses before and during meals [164]. Theoretically, this will result in more even mixing and gastric emptying of enzymes, though this has not been clinically proven. Enzymes are not required with simple carbohydrates

TABLE 42.5 Important Aspects of Early Care for Newly Diagnosed Infants and Young Children with CF

PERT (for PI)
High-calorie and high-fat diet (after human milk)
Fat-soluble vitamin supplementation (vitamins A, D, E, and K)
Salt supplements (essential to prevent fatalities)
Infection control (prevent all risky exposures)
Respiratory cultures (by vigorous oropharyngeal technique)
Antibiotic therapy as needed (goal: eradicate *Pseudomonas*)
Airway clearance teaching and recommendations
CF education with genetic counseling (CFTR genotype)
Lifestyle counseling (promote normal/quality life)

Source: Adapted from D. Borowitz, K.A. Robinson, M. Rosenfeld, S. D. Davis, K.A. Sabadosa, S.L. Spear, et al., Cystic fibrosis foundation evidence-based guidelines for management of infants with cystic fibrosis, J. Pediatr. 155 (2009) S73–S93.

(i.e., hard candy, popsicles, pop, jello) but are needed for foods containing fat, protein, and starch (rice, potatoes, etc.).

c Adjunctive Therapy

Histamine (H_2) antagonists and proton pump inhibitors inhibit gastric acid and in some cases may improve enzyme activity either by decreasing gastric acidity resulting in less destruction of unprotected conventional powder enzymes in the stomach, or by increasing pH in the upper intestine allowing for more rapid dissolution of the enteric coating dissolution and optimal conditions for enzymes to catalyze nutrients. As mentioned, efficacy of this treatment is not clear, as results in one study proved proton pump inhibitors to be helpful [159], while another found no benefit [160]. The CF caregiver should be cautioned that there are no safety data on the long-term use of these medications in children.

Newly Diagnosed Infants and Young Children up to 2 Years of Age

Guidelines for the care of infants diagnosed early through NBS were published in 2009 by Borowitz et al. [116]; key components of early care are summarized in Table 42.5. The core objectives of CF treatment after early diagnosis through NBS are to prevent malnutrition, control respiratory infections, and promote mucus clearance [116].

3 Initial Visits and Coordination with Primary Care Physician

The majority of young infants diagnosed through NBS appear to be totally healthy to the parents, and the diagnosis of CF is largely unexpected. Therefore, the psychosocial impact on the family must be carefully addressed at the initial visits [116]. Newly diagnosed infants with CF should be treated at an accredited CF center, ideally within 24–72 hours of diagnosis. At the first visit, adequate time for the family to receive comprehensive education and counseling is very important. Disbelief, anger, or anxiety about the new diagnosis is likely to be present, which affects the retention of information. Giving basic information in the clearest of terms and conveying the information in a sensitive, empathetic, and positive manner are key components of the visit. A variety of formats should be used to provide information, including verbal, written, and audiovisual methods.

Introduction of other members of the CF care team, namely the nurse, dietitian, respiratory therapist, and social worker, should occur with the first two visits [116]. This allows key components of nutrition and airway clearance to be taught, and facilitates the development of relationships with team members. A genetic counselor should meet with the family within 2 months of diagnosis to discuss in greater depth how mutations in the CFTR gene cause CF and the implications for other family members [116]. Equally important, the positive outlook for newly diagnosed infants should be reinforced and instill a sense of hope.

The pivotal role that both parents and primary care provider play as part of the CF team should be emphasized at the early visits [116]. Coordination with the primary care physician is essential, as families will be making numerous visits to their primary care provider and CF center during the first 2 years of life. Therefore, regular and open trilateral communications among the family, the primary care physician, and the CF center should be established. Communication between the primary care physician and the CF center is critical to ensure parents do not get conflicting messages, since many CF care goals are different from those of standard pediatric care (e.g., an emphasis on the need for the CF child to be chubby vs concerns about obesity in the general pediatric population).

4 Types of Feeding

Special attention to growth and nutrition early in life is essential because it is a time of extraordinary metabolic need; healthy infants double their birth weight by 4–6 months of age and triple it by 1 year. The first 6 months of life represent a unique window of opportunity to promote optimal growth, while poor growth during this critical period may be irreversible [39]. The U.S. CFF recommends that children reach a weight-for-length status of the 50th percentile by 2 years of age, with an emphasis of achieving this goal early in infancy [83,116]. However, optimal nutritional care to achieve this goal has not been defined.

a Human Milk Versus Formula

The basic principles of infant feeding for healthy term babies apply to feeding infants with CF. However, optimal feeding (i.e., breast milk, formula, or combination) to meet the increased nutritional requirement for infants with CF is unknown. The benefits of breastfeeding for healthy infants are widely recognized [165]. However, breast milk may be nutritional inadequate in calories, protein, EFA, and sodium to meet the increased requirements of CF infants, especially for those with MI or PI, who are at greater risk of poor growth and malnutrition [7,90,91,166–169]. On the other hand, breast milk's antimicrobial constituents are likely to offer protection against respiratory infections [170–173]. The issue of breastfeeding was less relevant before nationwide implementation of NBS, when CF infants were diagnosed at a median age of 8–9 months [174], an age when most infants would no longer be breastfed; now, the issue of breastfeeding is of prime importance.

Breastfeeding is historically discouraged for CF infants because of concerns about protein energy malnutrition, which is manifested by hypoproteinemia, hyponatremia, edema, and anemia [7,90,166–169]. Very few studies have examined whether exclusive breastfeeding promotes optimal growth and provides respiratory benefits for CF infants, and the findings are inconsistent due to various breastfeeding classifications, small sample size, and retrospective study designs [175–178]. The 2009 CFF infant care guidelines [116] continued its 2002 recommendation [82] to suggest breast milk as the initial type of feeding for CF infants, without specifying the exclusiveness or the duration of breastfeeding. As of 2013, approximately 60% of infants with CF were ever breastfed, and 15% were exclusively breastfed for at least 4 months [14]. A higher breastfeeding rate was observed in a recently launched multicenter observational study designed to evaluate the benefits and risks of breastfeeding in CF [179]. With that, more results will be generated to address issues related to breastfeeding in CF.

b Standard Formula Versus Special Formula

There is limited evidence to address whether formula-fed infants with CF and PI should consume special formula, for example, predigested formula containing protein hydrolysates and/or medium-chain triglycerides (MCTs). Among the three, very old studies conducted in the 1980s and 1990s, one reported similar nutritional status between CF infants fed hydrolyzed and standard formulas [180], another showed better anthropometric measures in infants fed hydrolysates [181], and the other study found improved fat and nitrogen absorption in infants fed semielemental formula when PERT was not given [182]. These conflicting results led the CFF to conclude insufficient evidence to recommend special formula for formula-fed infants with CF [116].

It is also unclear whether breast milk and standard formula should be routinely fortified to increase caloric and nutrient densities for feeding infants with CF who are growing adequately, for the purpose of sustaining normal growth or preventing growth faltering. This is another urgent nutritional issue recommended by the CFF for future research [116].

c Complimentary Foods

Infants with CF should be introduced to solid foods at the same age as healthy non-CF children, that is, 4–6 months of life, according to recommendations from the American Academy of Pediatrics. Nutrient and caloric-dense foods, such as meat, that will enhance weight gain and provide a good source of iron and zinc [141], are ideal as first foods for infants with CF. Breast milk or formula should continue through the first year of life, thereafter, whole cow's milk can be used in the thriving child.

As infants are introduced to table foods, it is important that families and primary care physicians understand that most children with CF need a balanced diet that is moderately high in fat to meet their nutritional requirement, which is different from the usual nutritional education given to families with healthy non-CF children for overweight and obesity prevention. For example, families should buy whole milk for the child with CF and lower fat milk for other children. During the second year of life, children establish self-feeding skills, food preferences, and dietary habits. Dietitians caring for children with CF should inquire about feeding behaviors to promote positive interactions and to prevent negative behaviors.

5 Enzyme Dose and Administration

PERT should be given with breast milk and formulas, including elemental and MCTs-containing formulas and all foods. An initial dose of 2000–5000 lipase units for each 120 mL feeding is recommended [116]. As the infant grows and the volume of intake increases, adjust the dose to up to 2500 lipase units per kg body weight per feeding, but not exceeding a maximal daily dose of 10,000 lipase units per kg body weight per day [116]. Enzyme dose in relation to caloric/fat intake and weight gain should be evaluated at each visit. The goal is to prescribe enzyme doses that are sufficient but not excessive to support optimal weight gain while minimizing the risk of fibrosing colonopathy. Nevertheless, caution to avoid fibrosing colonopathy may lead to excessively conservative enzyme dosing, as revealed from the CFF registry data that

TABLE 42.6 Recommendations for Daily Fat-Soluble Vitamin Supplementation[a]

	Vitamin A (IU)	Vitamin E (IU)	Vitamin D (IU)	Vitamin K (mg)
0–12 months	1500	40–50	400	0.3–0.5[a]
1–3 years	5000	80–150	400–800	0.3–0.5[a]
4–8 years	5000–10,000	100–200	400–800	0.3–0.5[a]
>8 years	10,000	200–400	400–800	0.3–0.5[a]

[a]*Currently, commercially available products do not have ideal doses for supplementation. Prothrombin time or, ideally, PIVKA-II levels should be checked in patients with liver disease, and vitamin K dose titrated as indicated.*
Sources: D. Borowitz, R.D. Baker, V. Stallings, Consensus report on nutrition for pediatric patients with cystic fibrosis, J. Pediatr. Gastroenterol. Nutr. 35 (2002) 246–259; D. Borowitz, K.A. Robinson, M. Rosenfeld, S.D. Davis, K.A. Sabadosa, S.L. Spear, et al., Cystic fibrosis foundation evidence-based guidelines for management of infants with cystic fibrosis, J. Pediatr. 155 (2009) S73–S93.

average enzyme dose tended to be at the low end of weight-based dosing early in life.

In infants with CF, PERT should be offered before feeding, mixed with 2–3 mL (1/2 tsp) applesauce, and given by spoon [116]. Other strained fruit can be used if applesauce is not taken, but parents should be encouraged to use only one type of food to avoid problems with potential food refusal if many different types of food are used as the vehicle for enzyme delivery.

After 1 year of age, children can be offered enteric-coated products, mixed with one food. Swallowing of capsules is encouraged as soon as parents consider the child is ready. This varies considerably from patient to patient but occurs usually around 4–5 years of age. If children continue to experience difficulties swallowing capsules, parents should open the capsule and sprinkle the beads in the mouth which can be ingested by drinking a liquid. Children should be discouraged from chewing the capsules, as this will destroy the protective coating of enzymes and may cause irritation in mouth.

6 Energy Intake and Nutrient Supplementation

Sufficient calories are critical in infants with CF, and the best indicators that energy requirement is met are maintenance of normal growth or achievement of catch-up growth. In addition to energy intake, adequate intakes of EFA and micronutrients such as zinc and sodium are needed to promote normal growth.

All infants with CF should receive standard, age-appropriate nonfat-soluble vitamins plus fat-soluble vitamins A, D, E, and K as recommended by the CFF guidelines (Table 42.6). Because of increased risk of hyponatremia, sodium supplementation is especially important in infants with CF [116], particularly in those fed human milk, which contains a very low amount of sodium. Older infants receiving solid foods are likely to have low sodium intake, as baby foods contain no added salt. CF infants younger than 6 months should receive a daily dose of one-eighth teaspoon of table salt; this amount should be increased to a quarter of teaspoon for older infants aged 6–12 months [116].

B Routine Management for Patients Older than 2 Years of Age

1 Energy Intake and Nutrient Supplementation

To obtain adequate energy intake and compensate for fat malabsorption, CF patients typically require a greater fat intake (35–40% of calories) than what is normally recommended for the general population (25–35%). Fat restriction is not recommended, because fat is the most energy-dense macronutrient and provides EFA. MCT supplements may be utilized as a good source of fat because they require less lipase activity, less bile salt for solubilization, and can be transported as free fatty acids through the portal system.

In CF patients, vitamin supplementation is necessary to prevent the occurrence of deficiencies. A standard, age-appropriate multivitamin supplement should be given to all CF patients. Additional supplementation with fat-soluble vitamins is needed (Table 42.6).

CF patients are at risk of hyponatremia because of salt loss through the skin. Children and adults are advised to consume a high-salt diet, especially during summer months and for those who live in hot climates.

2 Age-Specific Recommendations

a Preschool Age (2–5 Years)

CFF published its first clinical practice guidelines for preschoolers with CF in 2016 to address the gap for care for this age group [183]. This period is crucial for a child's growth and development, and interventions during this time will optimize nutritional status and preserve lung health [183]. Routine nutritional monitoring and care recommended by the preschoolers guidelines are summarized in Table 42.2. For children at nutritional risk, that is, BMI < 50th percentile, or rate of weight gain <50th

percentile expected for age, or weight-for-age < 10th percentile, the guidelines recommended more intensive management and frequent follow-up, that is, 8 weeks or sooner, to ensure normal growth. Issues related to behavioral assessment and management of mealtime are also addressed.

Children in this age group have developed self-feeding skills, food preferences, and dietary habits. Food intake and physical activity vary from day to day. For these reasons, close monitoring of dietary habits, caloric intake, and growth velocity are important. Routinely adding calories to table foods, in the form of additional fat, may help with maintaining optimal growth at this stage. The importance of serving calorie-dense foods (such as whole milk rather than low-fat milk) and establishing positive mealtime interactions should be emphasized.

Studies showed that toddlers with CF have longer meal times than their peers without CF, yet still do not meet the CFF's dietary recommendations for increased energy intake [184]. As the duration of meal times increases, difficult behaviors also tend to occur more frequently [185]. Therefore, dietary counseling should include assessment of eating behaviors. One strategy to address behavioral problems is to limit mealtimes to 15 minutes for toddlers and use snack times as mini-meals. Another strategy is to teach parents alternative ways of responding to their child who eats slowly or negotiates what he or she will eat.

b School Age (6–10 Years)

Children in this age group are at risk for declining growth for various reasons. They typically participate in a variety of activities, leading to limited time for meals and snacks. They are also exposed to peer pressure and challenged to begin self-managing their disease. These factors may affect compliance with prescribed medications such as pancreatic enzymes and fat-soluble vitamins. In addition, acceptance and understanding by teachers and fellow students may be lacking, further stressing a child with CF. Encouraging children to help in meal planning and preparation maybe helpful in improving food intake.

It is important to begin monitoring bone health at this age [125]. Bone health can be evaluated by history (atraumatic bone fracture), physical examination (poor growth, back pain), and by radiologic and laboratory assessment. According to current guidelines [125], CF children age 8 and older who are at risk for poor bone health (i.e., poor growth, poor lung function, history of bone fracture, delayed puberty, or chronic use of glucocorticoids) should be screened by dual energy X-ray absorptiometry to assess their bone mass. In addition, serum calcium, phosphorus, and 25-hydroxy-vitamin D and parathyroid hormone should be measured annually [125].

c Adolescence (10–18 Years)

This stage represents another vulnerable period of developing malnutrition because of increased nutritional requirements associated with accelerated growth, endocrine development, and high levels of physical activity. In addition, pulmonary disease often becomes more severe in this period, increasing energy requirement. This is also the age when other complications, such as CFRD, begin to occur more frequently, which further increases the risk of poor growth and malnutrition.

Puberty is often delayed in adolescents with CF; it usually is related to growth failure and poor nutritional status, rather than to a primary endocrine disorder. Assessment of puberty should be performed annually at 9 years of age in girls and 10 in boys by a standardized self-assessment or physician examination [186,187]. In addition to plotting growth on the growth charts, evaluating height and weight velocity [186] in association with Tanner stages can be very useful in identifying delayed or attenuated pubertal growth.

Nutritional counseling should be directed toward the patient rather than the parents. Teenagers may be more receptive to efforts to improve muscular strength and body image as a justification for better nutrition compared to emphasis on weight gain and improved disease status.

d Adulthood

CF patients reaching adulthood are usually responsible for the entire management of their disease, as well as for the financial burden of a chronic illness [188,189]. While in college or working, adults with CF are constantly adapting to new schedules and stresses. The goal of nutrition management is to maintain optimal BMI and to prevent unintentional weight loss. Nutritional counseling must be practical and pragmatic to help adults with CF adjust to these changes. A minimum of one comprehensive evaluation per year is recommended [188]. However, more specific recommendations are needed for adult CF clinical care.

e Pregnancy and Lactation

Widespread experience in recent years has demonstrated that pregnancy and lactation can be accomplished successfully by some women with CF. Pregnant women with CF should follow the guidelines from the Dietary Reference Intakes [190] for nutrient intakes. Special attention should be given to appropriate weight gain, particularly during the last trimester of the pregnancy. In addition to the usual multivitamin supplementation for CF, a prenatal vitamin should be consumed daily. During lactation, marked increase in caloric intake is necessary to meet the high energy requirement during this period.

TABLE 42.7 Maximizing Calories for Healthy Patients with CF

Adding Calories to Foods	High-Calorie Foods and Snacks[a]
• Add fats such as butter, gravy, cheese, or dressings to starches, fruits, and vegetables • Use whipped cream on fruits and desserts • Makes "super" milk: ½ cup whole milk + ½ cup half and half • Flavor milk with syrups or powders (chocolate, strawberry, etc.) or add whole milk yogurt to milk • Add eggs to hamburger meat or casseroles (never serve raw eggs) • Use extra salad dressing; avoid low-calorie or reduced calorie dressings • Serve gravies and cheese sauces	• Full fat ice cream, puddings • Cookies and milk • Cheese or peanut butter crackers • Muffins or bagels with cream cheese or butter • Cheese breadsticks • Chips and dip • French fries • Whole milk yogurt • Egg salad, tuna salad, cheese, or avocado slices with crackers • Trail mixes, nuts, and granola (after the age of 2 years) • Cold cuts, pizza • Fresh vegetables with salad dressing or dip

[a]*Assess age-appropriateness, especially with respect to choking risk in young children, before recommending.*

Source: Adapted from D. Borowitz, R.D. Baker, V. Stallings, Consensus report on nutrition for pediatric patients with cystic fibrosis, J. Pediatr. Gastroenterol. Nutr. 35 (2002) 246–259.

C Nutrition Intervention for Poor Growth and Malnutrition

For CF patients who are experiencing poor growth and malnutrition, nutritional intervention beyond the level of routine management is required. Nutrition support can be delivered at various levels, beginning from behavioral intervention, dietary modification, oral supplementation, to enteral or parenteral supplementation. In addition, the presence of comorbid medical conditions that is likely to affect growth and nutritional status, such as GERD, DIOS, and CFRD, should be evaluated.

1 Behavioral Intervention

In an effort to increase dietary intakes, caregivers of young children with CF may be engaged in ineffective feeding practices such as coaxing, commanding, physical prompts, and parental feeding. Adolescents with CF may intentionally skip pancreatic enzymes in order to achieve a certain body image. An in-depth assessment of eating behavior, feeding patterns, and family interactions at mealtimes should be performed in CF patients at risk or experiencing malnutrition. If negative behaviors are present, behavioral intervention should be used in conjunction with dietary intervention to improve intake. For example, one behavioral strategy is to gradually increase calories by working on one meal at a time. Another strategy is to teach parents alternative ways of responding to their child who eats slowly or negotiates what he or she will eat. Referral for more in-depth behavioral therapy is also encouraged as needed.

2 Dietary Intervention

a Oral Supplements

For infants experiencing inadequate weight gain, increasing caloric density of the feedings is the first step. This can be achieved by fortifying breast milk or by concentrating formula. For infants who are taking solids, additional calories can be added to infant cereal with the addition of carbohydrate polymers (e.g., Polycose) and/or fats (e.g., vegetable oil, MCT oil, or Microlipids).

Dietary intervention should begin with dietary modification to increase caloric density of the diet, that is, addition of high-calorie foods to the family's regular diet without dramatically increasing the amount of food consumed. For example, margarine or butter may be added to many foods, and half and half can be used in place of skim milk or water when preparing canned soup. More examples of how to maximize the caloric density of the diet are given in Table 42.7. If dietary modification is ineffective, use of energy supplement may be introduced. However, it is important to assure that the energy supplement is not used as a substitute for normal food intake.

b Enteral Feedings

Enteral feeding can be initiated when oral supplementation does not improve growth and nutritional status significantly. The goals of enteral feeding should be explained to the patient and family, that is, as a supportive therapy to improve quality of life and outcome, and their acceptance and commitment to this intervention should be realistically assessed.

Enteral feeding can be delivered via nasogastric tubes, gastrostomy tubes, and jejunostomy tubes. The choice of enterostomy tube and technique for its placement should be based on the expertise of the CF center. Nasogastric tubes are appropriate for short-term nutritional support in highly motivated patients. Gastrostomy tubes are more appropriate for patients who need long-term enteral nutrition. Jejunostomy tubes may be indicated in patients with severe GERD; use of predigested or elemental formula may be needed with jejunostomy feeding.

Standard enteral feeding formulas (complete protein, long-chain fat) are typically well tolerated. Calorically dense formulas (1.5−2.0 kcal/mL) are usually required to provide adequate energy. Nocturnal infusion is encouraged to promote normal eating patterns during the day. Initially, 30−50% of EER may be provided overnight. Pancreatic enzymes should be given with enteral feeding. However, optimal dosing regimen is unclear with overnight feeding.

3 Evaluation of Comorbid Medical Conditions

a Severe Malabsorption

A large number of patients with CF continue to have malabsorption despite adequate dosing with potent pancreatic enzymes [156]. Subjective symptoms such as abdominal bloating or cramps, or bulky stools cannot reliably assess the severity of malabsorption [69]. Instead, objective assessment is advocated by a 72-hour fat collection (while eating a regular diet) and the prescribed dose of enzymes. If severe fat malabsorption is identified (fecal fat losses exceeding 20% of intake) and is clearly contributing to abdominal symptoms or malnutrition, the dose of enzymes could be increased up to the maximum recommended amount. Alternatively, inhibition of gastric acid secretion with a histamine antagonist or a proton pump inhibitor may raise intestinal pH and improve the efficacy of enzyme therapy. Several weeks after the adjustment to therapy has been made, the individual patient should be reassessed by a repeat 72-hour fecal fat collection.

b Gastroesophageal Reflux Disease

GERD is quite common in CF infants [191], particularly in those with respiratory disease. Drugs to suppress gastric acid may be indicated if reflux is severe. A predigested formula offers no advantage and should be only considered in individuals who have had significant bowel resection following complicated MI [180].

c Distal Intestinal Obstruction Syndrome

DIOS is unique to CF [192]. It is characterized by cramping abdominal pain, which may be periumbilical or in the right lower quadrant. A mass is usually palpable in the ileocecal area. It should be emphasized that simple constipation is a common problem in individuals with CF. Consequently, a careful history, abdominal examination and abdominal X-ray, is indicated when abdominal pain due to DIOS is suspected in order to distinguish it from constipation and other CF-associated complications such as intussusception and appendiceal abscess.

DIOS is treated with several different approaches. If the DIOS is severe, a balanced electrolyte solution (used for cleansing the bowel prior to colonoscopy) is very effective in relieving the subacute obstruction. Complete bowel obstruction is an absolute contraindication to the use of these solutions. Volumes of 4−8 L, delivered at 1 L/hour are usually required for a complete clean out in children >10 years of age. In younger children, the electrolyte solution should be administered at a rate of 10−40 mL/kg body weight/hour for 4−6 hours until the stools no longer have any solid material. *N*-acetylcysteine and, in severe cases, large volume enemas with hyperosmolar contrast agents are also used. More recently, a polyethylene glycol solution without electrolytes has been used by some practitioners to help with the management of DIOS and/or constipation in CF. Anecdotal reports suggest that this solution, a powder mixed with any choice of beverage, at doses of about 17−34 g once or twice per day is effective in children with CF. Most patients who have an episode of DIOS are prone to have recurrent episodes and it is logical for these patients to maintain a bowel regimen using polyethylene glycol, although there are no published studies [191].

d CF-Related Diabetes

Adolescents and adults with CF and PI are at increased risk of developing CFRD. In many instances, patients exhibit no clear-cut signs and symptoms of diabetes. Furthermore, determination of hemoglobin A1C is not a reliable test for the diagnosis of CFRD. The diagnosis should be considered in any patient who is exhibiting weight loss or poor weight gain. In 2010, the CFF recommended annual screening for CFRD by a modified oral glucose tolerance test after the age of 10 years [17,18]. In the patient who has CFRD, high energy meals and snacks are encouraged but energy needs and insulin requirements must be carefully balanced. Foods high in simple sugars may be limited according to insulin needs. Multidisciplinary care and the support of endocrinologists and certified diabetes educators are essential. In individuals who have impaired glucose tolerance, close monitoring by both the CF and endocrine teams are required, as these patients are at increased risk of developing CFRD.

Other complications secondary to CF are also common, including hepatobiliary manifestations, bone/joint

disease, pancreatitis, small intestinal bacteria overgrowth, gut dysbiosis, anxiety disorder/depression, etc. Ongoing and careful management of these complications is essential for high-quality care to improve morbidity and quality of life in CF.

D Patient and Parent Education

Education of patients and their caregivers is a vital and routine component of the multidisciplinary care of patients with CF [82,116,187]. A solid grounding in the special nutritional needs of a patient with CF should be established at diagnosis. This should include an explanation of the role of the pancreas and how enzyme replacement therapy helps to improve maldigestion. Parents should be given specific instructions on how to provide an appetizing, high energy, nutritionally balanced diet, particularly with a liberal use of fat to provide extra calories. It is important to communicate the expectation that most children with CF are able to grow and gain weight normally. Patients and their parents require education about the importance of fat-soluble vitamins. Details on when to administer enzymes and vitamins must be reviewed on several occasions. In the older children, concerns about adherence should be emphasized and assessed at each follow-up visit.

V CONCLUSIONS

The clear associations between nutritional status and clinical outcomes in CF mandate careful nutritional assessment, management, and monitoring of all patients with CF. In recent years, with new knowledge arising from NBS research, there has been a shift away from the idea that malnutrition is inevitable for most CF patients toward the more optimistic view that adequate nutrition and growth are possible if early diagnosis and aggressive nutritional monitoring and therapy are made for each individual patient. This task is best accomplished by involving a multidisciplinary team that includes dietitians in the care and management of CF patients. In this way, the goals of normal growth and prevention of malnutrition can be attained, which will improve the prognosis and quality of life for patients with CF.

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Chapter 43

Current Understanding of Vitamin D Metabolism, Nutritional Status, and Role in Disease Prevention

Susan J. Whiting[1], **Mona S. Calvo**[2] **and Hassan Vatanparast**[1]

[1]*University of Saskatchewan, Saskatoon, SK, Canada,* [2]*Center for Food Safety and Applied Nutrition, Food and Drug Administration, Laurel, MD, United States*

I INTRODUCTION

Vitamin D is a nutrient that, until recently, was neglected by the nutrition community. Although recognized in the early 20th century as an essential nutrient, recommendations for intake were often qualified as being needed only in the absence of sunlight. In theory (and in ancient times when early humans all lived closer to the equator), all vitamin D needs could be met by exposure to sunlight that provided ultraviolet (UV) B radiation, but only recently have we come to understand how UVB acts and what other factors—particularly environmental—mitigate cutaneous vitamin D synthesis. Studying vitamin D requirements is difficult. Early dietary recommendations for vitamin D, such as the 1989 Recommended Dietary Allowance (RDA) [1], indicated a "relative paucity of recent controlled studies [and] ... lack of data on which to base requirements." It further stated that "clinical osteomalacia appears to be rare in the United States." What is known today, however, is that vitamin D deficiency and insufficiency are widespread [2], which was not identified when the Dietary Reference Intakes (DRIs) for vitamin D were first published in 1997 [3]. In 2011, new DRIs for vitamin D reflected the need for more dietary vitamin D [4]. While the 2011 DRI report did not set recommendations based on functions other than bone health, there remains a growing body of evidence for vitamin D's many roles in the body [5,6].

The role of vitamin D in preventing rickets was discovered early in the 19th century but it was not until the 1970s that the sequence of steps from skin precursors to active metabolite was understood. Despite the interest generated in solving the puzzle of how vitamin D increased intestinal calcium absorption, there were several reasons why progress toward a better understanding of vitamin D requirements failed to progress. There were technical challenges in analyzing vitamin D and its metabolites. Beginning in the 1980s, there was a greater focus on dietary calcium as the major "bone" nutrient, leaving vitamin D with only a minor role in osteoporosis research. And finally, the important contribution of sun exposure to vitamin D status was not fully realized until recently. Indeed, dietary intake recommendations for situations of complete year-round lack of skin synthesis give values that are 5−8 times higher than those recommended to maintain vitamin D status only through the winter. It has been shown that globally there is greater prevalence of chronic diseases such as cancer and immune disorders at extremes of latitudes where sun exposure for skin synthesis of vitamin D is limited [5].

Vitamin D affects people starting with fetal development and continuing to old age, functioning at both the genomic and nongenomic level in the regulation of key protein synthesis or in the intracellular metabolic pathways in virtually all tissues [5,6]. Growth, development, and maintenance of health are all affected, and in many regards, quality of life is as well. This chapter, while acknowledging vitamin D's contribution through the life span, focuses on vitamin D needs for maintenance of health, and on vitamin D's specific actions in selected clinical conditions. As research is ongoing, the reader can

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expect to learn enough about vitamin D's roles to be able to understand and apply the research as it unfolds.

II METABOLISM OF VITAMIN D

A Overview of Vitamin D Synthesis and Conversion to Its Active Metabolite

1 Vitamin D Is a Family of Compounds

To understand vitamin D is to appreciate the functions of the numerous metabolites that arise during its metabolism. Fig. 43.1 shows how vitamin D is provided, either through skin synthesis or from diet, and undergoes successive hydroxylations to form the active metabolite, 1,25-dihydroxyvitamin D. There are several natural occurring forms of vitamin D and many metabolites, and these are outlined in Table 43.1. The term "vitamin D" really represents all compounds having or potentially having the activity that we associate with the active metabolite of vitamin D; however, in nutrition we also refer to the precursor molecules provided in the diet or in supplements, which are cholecalciferol (for vitamin D_3) and ergocalciferol (for vitamin D_2), as "vitamin D." Generic use of the term "vitamin D" is a source of great confusion. Through this chapter, an attempt has been made to use the exact term for each metabolite in order to prevent confusion. Table 43.1 can be used as a guide for this purpose. When vitamin D_2 and D_3 can contribute to the same function, then "vitamin D" with no subscript is used.

2 Vitamin D_3 Synthesis in Skin

In the skin, there is 7-dehydrocholesterol (also called "provitamin D_3") in the epidermis and the dermis, which reacts, when UVB radiation in the wavelength range of 280–315 nm passes through these skin layers, to form previtamin D_3. Previtamin D_3 forms rapidly, however, skin pigmentation (melanin) competes with 7-dehydrocholesterol for the UVB photons, and therefore reduces the amount of UVB that can act on 7-dehydrocholesterol to form previtamin D_3. With prolonged exposure to UVB, inactive compounds are formed instead of previtamin D_3. Over a prolonged period of time, the previtamin D_3 that is formed

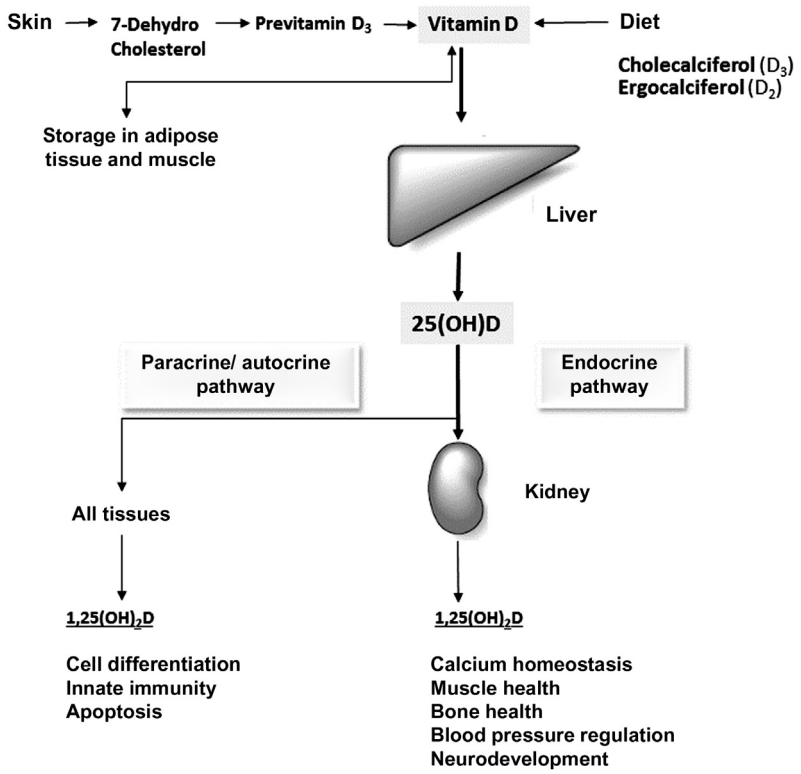

FIGURE 43.1 Overview of vitamin D metabolism, from synthesis (or provision in the diet) through to synthesis of active form. Once vitamin D_3 is made in skin, or provided from the diet (and some of this could be as vitamin D_2), it is converted in the liver to 25-hydroxyvitamin D [25(OH)D], which is the major circulating form of vitamin D. This 25(OH)D is now the substrate for production of the active form, 1,25-dihydroxyvitamin D, via two pathways. In the endocrine pathway, 1,25-dihydroxyvitamin D is made in the kidney under tight regulatory control; this 1,25-dihydroxyvitamin D circulates in the blood and acts to promote active calcium and phosphate absorption; working with PTH, the active form affects bone metabolism and kidney reabsorption of calcium. In the paracrine/autocrine pathway, 1,25-dihydroxyvitamin D is made and used locally by a variety of cells, including those of the immune system. *Modified from B.W. Hollis, C.L Wagner, Nutritional vitamin D status during pregnancy: reasons for concern, Can. Med. Assoc. J. 174 (2006) 1287–1290.*

TABLE 43.1 A Glossary of Vitamin D Compounds and Metabolites

Vitamin D Metabolite	Alternate Name(s)	Function	Clinical Utility of Measurement
"Vitamin D" (often used as synonym for vitamin D_3 or all dietary sources)	N/A	Term used to describe actions of the active metabolites of vitamins D_2 and D_3	N/A
7-Dehydrocholesterol	Provitamin D_3	Precursor to cholecalciferol found in skin; is acted upon by UVB	Not measured
Previtamin D_3	N/A	Intermediate in synthesis of cholecalciferol from 7-dehydrocholesterol	Not measured
Vitamin D_3	Cholecalciferol; calciol	Form of vitamin synthesized by animals in presence of UVB light	Provides information of recent sun exposure or ingestion of vitamin D_3
Vitamin D_2	Ergocalciferol	Form of vitamin synthesized by yeast and fungi in presence of UVB light	Provides information of ingestion of vitamin D_2
25-Hydroxyvitamin D_3 [25(OH)D_3]	25-Hydroxycholecalciferol; calcidiol, calcifediol	Circulating form of vitamin D_3, made from cholecalciferol	Measure of vitamin D_3 status
25-Hydroxyvitamin D_2 [25(OH)D_3]	25-Hydroxyergocalciferol	Circulating form of vitamin D_2, made from ergocalciferol	Measure of vitamin D_2 status
Total 25-hydroxyvitamin D [total 25(OH)D]	Total 25(OH)D	Represents the sum of 25(OH)D_2 + 25(OH)D_3	Measured using RIAs and other early assays and reflects all dietary and skin-derived sources
1,25-Dihydroxyvitamin D_3 [1,25-DHD]	1,25-Dihydroxycholecalciferol; calcitriol	Active metabolite of vitamin D_3	Measured to determine if active form can be made or to monitor treatment; not useful for vitamin D status
1,25-Dihydroxyvitamin D_2	1,25-Dihydroxyergocalciferol	Active metabolite of vitamin D_2	Measured to determine if active form can be made or to monitor treatment; not useful for vitamin D status
24,25-Dihydroxyvitamin D	24,25-Dihydroxycholecalciferol [or -ergocalciferol	Inactive; made instead of 1,25 metabolite if 1-hydroxylase is not stimulated	Not measured
1,24, 25-Trihydroxyvitamin D	Calcitroic acid	Inactive; made from 1,25 metabolite as mechanism for inactivation	Not measured
3-Epimers of vitamin D metabolites	3-Epi-25(OH)D_3	Unknown; found as a small percentage of total 25(OH)D in adults but in higher amounts in infants	Not routinely measured
	3-Epi-1,25$(OH)_2D_3$	Possibly has some activity	Not routinely measured

is changed due to thermal isomerization to vitamin D_3 (more appropriately called "cholecalciferol" or, less commonly, "calciol"). The reaction to form previtamin D_3 takes little time but the reaction converting previtamin D_3 to cholecalciferol, takes hours to occur [6] and is a rate-limiting step. Should more UVB photons reach the epidermis and dermis, previtamin D_3 is converted to inactive compounds with no vitamin D activity (tachysterol and luminsterol). Thus, excess exposure to UVB does not result in excess vitamin D production [6].

Besides the skin pigment melanin, other factors reduce skin synthesis of cholecalciferol. These include clothing (although some loosely woven clothing does permit UVB to pass through); window glass, sun screens formulated to

block UVB, particularly when the sun protection factor (SPF) is over 8 [6]; being indoors; tall buildings creating urban canyons; cloudy days; smog and light-blocking air pollution; and winter, when the sun does not rise far enough above the horizon to allow sufficient UVB irradiation to stimulate dermal vitamin D_3 synthesis. Thus, the term "vitamin D winter" refers to the time of year when UVB radiation is not sufficient for cholecalciferol synthesis in the skin.

Latitude is the major determining factor for intensity of UVB irradiation—whether in the southern or northern hemisphere. At the equator, vitamin D can be made year-round even in darker pigmented skin. Above latitude 37° there are 4 months of vitamin D winter; at latitude 42° there are 5 months, and close to the poles there would be no time during the year when vitamin D synthesis in the skin could occur [6,7]. Yet, not all analyses of vitamin D status and latitude show the expected relationship [8,9]. For example, there is now considerable vitamin D deficiency and insufficiency in people living near the equator, in countries of the Middle East, southern Europe [9], and in India [10]. This suggests that many factors are determining skin synthesis, so lack of UVB (e.g., "vitamin D winter") affects some countries, while clothing and customs such as time spent outdoors determine vitamin D in other countries. Age is another factor decreasing skin synthesis of cholecalciferol, as described below.

3 Conversion of Cholecalciferol and Ergocalciferol to 25-Hydroxyvitamin D

The cholecalciferol made from skin synthesis is released from the epidermis into the blood, where it is bound to vitamin D binding protein (DBP). Cholecalciferol and ergocalciferol (vitamin D_2) in the diet are absorbed and carried to the liver in chylomicrons. Intestinal absorption is not a limiting factor except when there is fat malabsorption (e.g., cystic fibrosis, Crohn's disease). Generally, fat-soluble vitamins are better absorbed with dietary fat. Added vitamin D_3 in orange juice is well absorbed despite just a small amount of fat in orange juice [11]; however, a study of a large (50,000 IU) dose showed 32% better absorption from a meal containing 30% fat compared to a fat-free meal [12]. Cholecalciferol and ergocalciferol circulate for only 1−2 days. This quick turnover is due to rapid hepatic conversion and uptake by fat and muscle cells [6,13].

There are two steps leading to the active form of vitamin D, which is 1,25-dihydroxyvitamin D (see Table 43.1). The first step is converting cholecalciferol or ergocalciferol to the major circulating form. This pathway involves four hepatic cytochrome P-450 enzymes (CYP2R1, CYP27A1, CYP3A4, CYP2J3) [6] that hydroxylate cholecalciferol or ergocalciferol at carbon 25. The resulting metabolites, 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 (together denoted as total 25(OH)D), are released into circulation. The amount of 25(OH)D that circulates is determined by the availability of its substrate, cholecalciferol or ergocalciferol [13]. With increasing levels of cholecalciferol made in skin or of dietary cholecalciferol and ergocalciferol, levels of 25(OH)D increase [6,14]. It is important to appreciate that 25(OH)D is the key metabolite indicating vitamin D status [6]. It is not the metabolically active form but it is the form that most accurately reflects deficiency or excess and is therefore used as a measure of vitamin D nutritional status [13].

Availability of serum 25(OH) is required for vitamin D activity. Fig. 43.2 illustrates the changes in 25(OH)D over a year, demonstrating how sun exposure impacts vitamin D status in light-skinned individuals living at about 50°N latitude [15]. One can see the impact of "vitamin D winter" in these subjects. This figure also demonstrates how an oral supplement of cholecalciferol can maintain the summer level of 25(OH)D through the winter months [15]. Studies indicate that while the molecule 25(OH)D has a half-life of 2−3 weeks [5], the amount in blood has an effective half-life of 2 months due to contribution from stores [16]. This means that in the absence of both

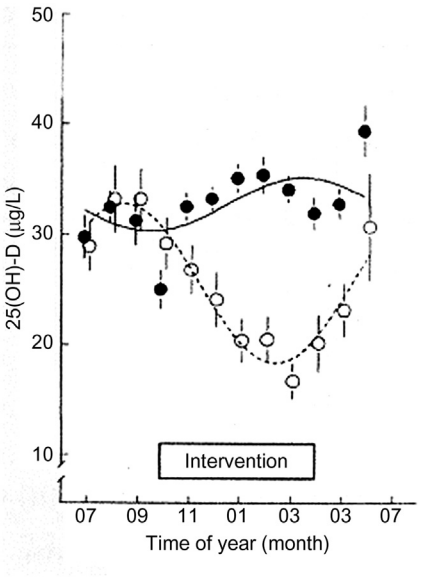

FIGURE 43.2 The data for this graph was derived from an RCT conducted in Germany (latitude 50°N). Open circles (○) represent mean levels of serum 25(OH)D of subjects throughout the year. Closed circles (●) are mean levels of serum 25(OH) in subjects who received the treatment regimen of 12.5 μg (500 IU) cholecalciferol and 500 mg calcium. Note that the units for 25(OH)D are expressed as μg/L (to convert to nmol/L, multiply by 2.5). *Reproduced with permission from C. Meier, H.W. Woitge, K. Witte, B. Lemmer, M.J. Seibel, Supplementation with oral vitamin D_3 and calcium during winter prevents seasonal bone loss: a randomized controlled open-label prospective trial, J. Bone Miner. Res. 19 (2004) 1221−1230.*

sun and adequate dietary source of cholecalciferol, serum levels of 25(OH)D will decline throughout the winter months.

Controversy surrounds the equivalency of the two precursors—cholecalciferol versus ergocalciferol—in the liver conversion to 25(OH)D [14,17]. When a single dose of either vitamin D_2 or vitamin D_3 was administered, $25(OH)D_2$ was shown to remain in blood for a much shorter time than a comparable dose of $25(OH)D_3$, declining after 1 week [18]. Nevertheless, when equal doses (1000 IU) of vitamin D_2 or vitamin D_3 were given daily to subjects, levels of 25(OH)D rose to the same extent, suggesting that physiologic amounts of either vitamin D form work similarly [19] when used on a daily basis as is typical of most dietary supplement use. What other studies find, however, is that providing vitamin D_2 results in production of $25(OH)D_2$ which is accompanied by a drop in $25(OH)D_3$ but little change in total 25(OH)D [14,17]. The health significance of this reduction in $25(OH)D_3$ with an increase in vitamin D_2 intake is unknown.

A study [13] looking at whether provision of the substrate for synthesis of 25(OH)D was rate-limiting, they reported a wide range of substrate levels in subjects given high doses of cholecalciferol or those living in a sunny near-equatorial region. As shown in Fig. 43.3, high doses of cholecalciferol from supplements (as much as 6400 IU) shown in panel A or sun exposure in Hawaii in panel B affected serum 25(OH)D levels similarly. The relationship is not linear, indicating a controlled, saturable reaction. Unless cholecalciferol is provided in sufficient amounts, the production of 25(OH)D is limited by its substrate.

4 Renal Conversion to the Active Metabolite: Endocrine Pathway of 1,25-Dihydroxyvitamin D Synthesis and Use

There are two pathways for conversion of 25-hydroxyvitamin D to the active form of vitamin D, which is 1,25-dihydroxyvitamin D (Fig. 43.1). The first to be described is the better known and understood pathway, now referred to as the endocrine pathway. In the endocrine pathway 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] is synthesized in one tissue but acts elsewhere, thus fulfilling the definition of a "hormone."

In proximal renal epithelial cells, 1,25-dihydroxyvitamin D is made when the enzyme 1-α hydroxylase (also called CYP27B1) is stimulated by parathyroid hormone (PTH) (which had previously been stimulated by a low circulating plasma calcium level) or by a fall in intracellular phosphate levels [6,20]. This is a tightly controlled endocrine system and considered to be the major contributor to the circulating levels of the active metabolite of vitamin D. Plasma levels of 1,25-dihydroxyvitamin D rise only when needed, as a result of synthesis in the kidney. The "need" for vitamin D action relates to its primary role (endocrine function) of providing the building blocks of bone—calcium and phosphate. A need for calcium, this is expressed as hypocalcemia, acts as the trigger for synthesis and release of PTH. An increase in PTH has three actions relating to calcium metabolism: (1) increased resorption of bone in order to provide immediate calcium to the blood; (2) more efficient reabsorption of calcium in the renal tubule, in order to conserve blood calcium; and (3) increased activity of the 1-hydroxylase in the proximal renal tubule to increase

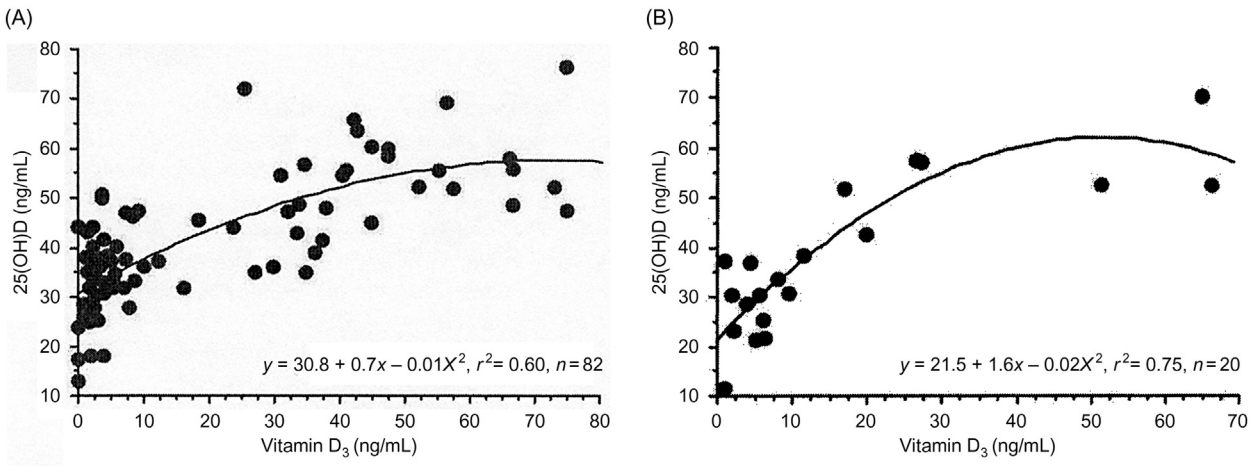

FIGURE 43.3 These graphs illustrate how either a high dose of cholecalciferol from a supplement of 150 μg (6000 IU) in panel A or sun exposure in Hawaii in panel B affect serum 25(OH)D. The authors of the study concluded that vitamin D status, as measured by serum 25(OH)D, is affected equally by diet or sun exposure. Further, unless cholecalciferol is provided in sufficient amounts, the production of 25(OH)D is limited by its substrate. *Reproduced with permission from B.W. Hollis, Circulating vitamin D3 and 25-hydroxyvitamin D in humans: an important tool to define adequate nutritional vitamin D status, J. Steroid. Biochem. Mol. Biol. 103 (2007) 631–634.*

levels of 1,25-dihydroxyvitamin D [21]. This third action, therefore, increases the concentration of 1,25-dihydroxyvitamin D in the blood that can travel to the small intestine and promote active absorption of calcium. The increase in plasma 1,25-dihydroxyvitamin D is also a way to provide this metabolite to other cells such as bone, parathyroid gland, and kidney, where it acts in concert with PTH to increase blood calcium levels. To complete the cycle, a rise in serum 1,25-dihydroxycholecalciferol acts to suppress PTH secretion. This type of regulation is a classical endocrine negative feedback mechanism.

Historically, the main action of circulating 1,25-dihydroxyvitamin D is to increase calcium (and phosphate) absorption [22]. This occurs in the duodenum, in enterocytes, where active calcium (and phosphate) absorption is promoted through genomic and nongenomic actions. The genomic mechanism of action of 1,25-dihydroxyvitamin D operates through receptors in the membrane of the cell nucleus, similar to that of other steroid hormones, while the nongenomic receptor is thought to operate through the cell's plasma membrane receptors. In either case, there is binding to a specific receptor called the vitamin D receptor (VDR) at the cell membrane of the enterocyte. In the nucleus, this complex, along with other coactivators, binds to a vitamin D response element (VDRE) to promote transcription of specific gene products. It has been long assumed that one protein product, a calcium binding protein called calbindin 9K, was required for translocation of calcium through the enterocyte during active transport of calcium (otherwise calcium absorption is passive, via paracellular channels) [5]. Studies with a knockout mouse having no gene for calbindin 9K showed that 1,25-dihydroxyvitamin D-mediated calcium absorption was normal in this mouse, thus leaving the exact gene products for active calcium translocation in doubt [23].

Without active absorption, the amount of calcium that can be absorbed is limited to about 15% of calcium intake which is paracellular (passive) absorption [24]. The effectiveness of passive absorption is dependent on the lumen concentration of calcium. When vitamin D status is improved, calcium absorption rises but eventually reaches a threshold level of about 30% in normal healthy adults who have no urgent need for calcium. In growth and pregnancy, calcium absorption can rise to levels of 80%, but only if vitamin D status is adequate; hormones, such as growth hormone and prolactin (respectively), are important for this high absorption value [3,5].

A second important action of circulating 1,25-dihydroxyvitamin D is to promote synthesis of mature osteoclasts [6]. Here, 1,25-dihydroxyvitamin D stimulates the osteoblasts to synthesize a specific receptor ligand, known as RANKL (receptor activator of the nuclear factor kappa-B ligand, abbreviated as NF-κB). These osteoblasts have the VDR, and the action of 1,25-dihydroxyvitamin D is genomic stimulating the RANKL protein synthesis. Once the RANKL is made, it exits the osteoblast and binds to a receptor (RANK) on preosteoclasts. This binding induces maturation of the cells into osteoclasts, which function to resorb bone. And the net result is release of calcium and phosphate into circulation. Thus, having been made in the kidney in response to a need for calcium or phosphate, 1,25-dihydroxyvitamin D's actions in the intestine and bone result in an increase of blood levels of both calcium and phosphorus.

5 Extra-Renal Conversion to the Active Metabolite: Paracrine/Autocrine Pathway of 1,25-Dihydroxyvitamin D Synthesis and Use

The other pathway for conversion of 25(OH)D to $1,25(OH)_2D$ is called the paracrine/autocrine pathway because these terms denote that the molecule is used locally in adjacent cells or used in the same cell in which it is made, respectively (Fig. 43.1). Less is known about this, but researchers found the 1-hydroxylase enzyme in many tissues other than the renal proximal tubule, and those working in cell culture systems were able to measure $1,25(OH)_2D$ production [25]. Further, the VDR has been identified in most tissues including brain, prostate, breast, gonads, colon, pancreas, heart, monocytes, and lymphocytes. Extra-renal $1,25(OH)_2D$ production is not regulated by serum calcium, phosphate, PTH, or other hormones such as fibroblast growth factor (FGF-23) released from bone [6]. Many actions have been proposed, but the modulation of immune function through actions on lymphocytes and macrophages is the most developed and seemingly has the greatest impact on chronic disease risk.

Cells of the immune system can produce $1,25(OH)_2D$ that acts in a paracrine manner to modulate the immune response [25–27]. For example, macrophages are an important part of the host defense against a variety of pathogenic organisms, including *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis. Macrophages are activated to kill such pathogens because pattern-recognition receptors, such as toll-like receptor (TLR) 1 and 2, recognize molecular patterns associated with microorganisms that are not found in mammalian cells. In the case of tuberculosis, macrophages are activated via recognition of *M. tuberculosis* lipopeptides by a TLR2/1 dimer. This interaction triggers antibacterial mechanisms of the macrophage, including the production of the antibacterial peptide cathelicidin, which can kill susceptible pathogens [26]. Cathelicidin transcription is enhanced by $1,25(OH)_2D$ acting via the VDR to increase gene transcription. This TLR2/1 interaction also stimulates the expression of the 1-hydroxylase enzyme which catalyzes $1,25(OH)_2D$ synthesis, suggesting that vitamin D deficiency may impair protection against tuberculosis by

limiting $1,25(OH)_2D$ production by macrophages, although human intervention trials are needed to test this hypothesis [26]. In addition to vitamin D stimulation of antimicrobial peptides, it functions in the modulation of proinflammatory and antiinflammatory cytokines and chemokines secreted in response to bacterial endotoxins, and viral and parasitic agents [25].

Vitamin D also regulates aspects of adaptive immunity. Adaptive immunity refers to the acquired immune response that develops in response to infections and vaccinations. This process not only disables infectious agents but also provides "memory" that subsequently prevents a person from getting most infections a second time. T lymphocytes are an important component of adaptive immunity functioning as helper cells which promote antibody production by B cells. T lymphocytes (natural killer cells) also act as effector cells to kill virus-infected host cells or to promote macrophage-mediated responses, such as the one described in the previous paragraph against *M. tuberculosis*. Another function of 1,25-dihydroxyvitamin D is to act as a modulator of the adaptive immune system, thus having a role in infectious and autoimmune disease in addition to its well-characterized function in innate immune response [25,27].

6 Mechanism of Action of the Active Metabolite

The active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2$ D), whether synthesized in the kidneys and released to the circulation or originating from extra-renal 1-alpha-hydroxylase activity is thought to operate through two distinctly different mechanisms: (1) the classical genomic action and (2) the rapid membrane initiated action or nongenomic action [28].

a Genomic Action

The classical genomic action of $1,25(OH)_2$ D involves the binding of this steroid hormone to its nuclear receptor which is a stereo-specific interaction. The vitamin D nuclear receptor is a member of the super family of steroid hormone nuclear receptors. Ligand-nuclear receptor binding initiates the cell's transcriptional machinery to regulate gene transcription. In the currently accepted model of $1,25(OH)_2D$ and VDR_{nuc} activation of gene transcription, when the ligand or 1,25(OH)D binds the nuclear receptor, it forms a heterodimer with the nuclear retinoid-X receptor. This heterodimer−DNA complex then interacts with the appropriate VDRE on the promoter genes of specific target cells which are up- or downregulated. The heterodimer−DNA complex then recruits necessary coactivator proteins to form a competent transcriptional complex capable of modulating mRNA production [28,29]. The discovery of the presence of the VDR_{nuc} in over 30 human tissues led to our new understanding of vitamin D's role in the regulation of B and T lymphocytes of the immune system, hair follicles, muscle, adipose tissue, bone marrow, and cancer cells through the mechanism of nuclear VDR regulation of gene transcription [29].

b Nongenomic Action

There are $1,25(OH)_2D$-mediated responses that were observed to occur within minutes to an hour; these actions were considered too rapid to be explained by the nuclear VDR regulating gene transcription mechanism. Such rapid responses included secretion of insulin by pancreatic beta-cells, rapid migration of endothelial cells, Ca^{2+} influx in skeletal muscle cells as modulated by phospholipase C, protein kinase C and tyrosine kinase, and activation of mitogen-activated kinase to identify only a few [28,29]. $1,25(OH)_2D$ can rapidly activate signal transduction pathways in addition to activation of the slower classical genomic mechanism of gene transcriptional regulation. Studies show that $1,25(OH)_2D$ can rapidly activate intracellular signaling molecules [30]. The plasma membrane receptor, termed membrane-associated rapid response steroid binding protein, does not behave as traditional membrane spanning receptors and can be found in the endoplasmic reticulum or relocated in the nucleus [30]. Specific VDR called VDR_m have been identified within the plasma membrane caveolae in a variety of different cell types including: intestine, kidney, lungs, leukemia, and osteoblast-like cells [29−31]. The nongenomic mechanisms may be critical for immune responses [30]; however, many important questions remain to be resolved concerning membrane-initiated vitamin D action.

B Inactivation and Excretion of Vitamin D

The hydroxylases in vitamin D metabolism are cytochrome P450 proteins. Some of these enzymes have been described above. There is the enzyme CYP24A1 also called 25-hydroxyvitamin D_3-24-hydroxylase which is responsible for catalyzing the conversion of 25(OH) D_3 and $1,25(OH)_2D_3$ into 24-hydroxylated products including $1,24,25(OH)_3D_3$ and 24,25 $(OH)_2D_3$, respectively (Fig. 43.4) [20,32]. This enzyme plays an important role in preventing unwanted buildup of 1,25-dihydroxyvitamin D by inactivating its precursor by forming the metabolite $1,24,25(OH)_3D_3$ that is also called calcitroic acid. Similar to other steroid hormones, most vitamin D metabolites are conjugated with gluconate or sulfate and excreted into the bile. Recent studies have shown a correlation between CYP24A1 and several diseases. Although the relationship between extensive

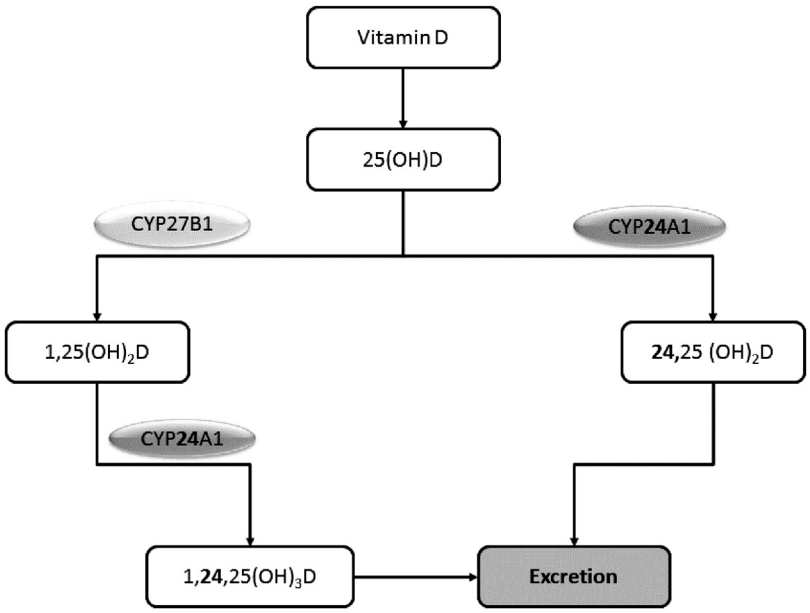

FIGURE 43.4 The pathway for inactivation of vitamin D involving conversion of either 25-hydroxyvitamin D [25(OH)D] or 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] by the enzyme CYP24. *From W.L. Miller, Genetic disorders of vitamin D biosynthesis and degradation, J. Steroid Biochem. Mol. Biol. 165 (2017) 101–108.*

accumulation of active vitamin D and idiopathic infantile hypercalcemia has been suspected for over 50 years, the actual cause of this vitamin D metabolism defect was not revealed until recently [32]. Now, the inactivating mutations of CYP24A1 are thought to be the cause of idiopathic infantile hypercalcemia. Further, the involvement of CYP24A1 in hypophosphatemia, chronic kidney diseases (CKDs), and hyperproliferative disorders (e.g., cancer, psoriasis) has encouraged scientists to develop CYP24A1 inhibitors and examine their effect in controlling vitamin D hypercatabolism [32].

III SOURCES OF VITAMIN D

A Food Sources of Cholecalciferol and Ergocalciferol

Vitamin D content in foods and supplements for either ergocalciferol or cholecalciferol are expressed as IU (international units) and in micrograms (μg), where 1 μg = 40 IU. Most food and supplement sources continue to use IU as the way to express content, so this convention is presented in this chapter. Data on food composition in Table 43.2 are divided into naturally occurring sources, fortified foods, and foods that have undergone bioaddition. There are only a few foods that naturally contain vitamin D as ergocalciferol or cholecalciferol [33] (Table 43.2), with fish having the greatest amount. In the wild, fish are part of a food chain that allows for concentration of vitamin D in the flesh of fatty fish (e.g., salmon, sardines, mackerel), while in lean fish, vitamin D is concentrated in liver (e.g., cod liver oil). With fish farming, levels of cholecalciferol in fish raised in aquaculture cannot be assumed to be equivalent to wild species. Further, it is now recognized that levels in fish are much more variable than previously recognized [34]; therefore, caution must be taken in using composition data. Other concerns about over-consumption of fish or fish oils include consumption of too much mercury and vitamin A. The muscle (meat) or liver of land animals that are exposed to sunlight or have vitamin D in their feed may be a natural source of vitamin D. Eggs are either a natural source of vitamin D, or can have additional vitamin D with bioaddition where the vitamin D is added to chicken feed [35]. Some foods such as meat and eggs also contain 25(OH)D which adds to the vitamin D content. "Potency adjusted" levels of 25(OH)D are the measured 25(OH)D levels multiplied by five [36,37].

Fortification provides close to three-quarters of the food-derived intake of vitamin D as measured in NHANES 2003–06 [38]. Most of the foods shown in Table 43.2 are fortified with vitamin D_3. In accord with a 2010 FDA regulation, soy products and other plant-based milks can be fortified with vitamin D_2 only [39]. Such foods can appeal to vegetarians who may prefer to consume a plant-based form of vitamin D. The use of a patented yeast which synthesizes vitamin D_2 after exposure to UVB light was recently approved in the United States and Europe for making bread and other baked goods. According to a recent study, Vitamin D_2 from light

TABLE 43.2 Selected Common Food Sources of Vitamin D in the United States

Food	Source of Vitamin D	Vitamin D_3 IU/serving	Potency Adjusted 25(OH)D IU/serving
Naturally Occurring			
Salmon, canned, 85 g (3 ounces)	Naturally occurring but variable	396–649	
Farmed salmon, 100 g[d]	Reduced due to use of plant protein-based foods	6.18 μg	
Sun dried shiitake mushrooms, 36 g (¼ cup cooked)	Naturally occurring; exposed to sun (UVB)	110 (vitamin D_2)	
Pork, cooked[b]	Naturally occurring but variable due to feed and/or sun exposure	31	50
Egg, 1 whole	Regular feed	40 (29–103[a])	88–106[a]
Fortified			
Fluid cow's milk, 250 mL (1 cup)	Fortification	100	
Orange juice with added calcium and vitamin D, 125 mL (1/2 cup)	Fortification of selected brands	50	
Plant-based milks such as soy or almond, 250 mL (1 cup)[a]	Fortification of selected brands	60–120 (vitamin D_2)	
Yogurt 170 g	Fortification of selected brands	60–200	
Cheese slice, 16 g	Fortification of selected brands	60	
Margarine, 10 g (2 teaspoons)	Fortification of selected brands	30–200	
Cereals, ready-to-eat, 1 serving (1/2 to ¾ cup)	Fortification of selected brands	40–100	
Bread, 100 g	Fortification of selected brands	90	
Bioaddition			
Portobello mushrooms, 85 g	Irradiated with UVB	400 as vitamin D_2	
Bread, 100 g	Use of irradiated yeast to make bread	400[c]	
Egg, whole[a]	Feed enhanced with vitamin D	280	180

[a] *J. Exler, K.M. Phillips, K.Y. Patterson, J.M. Holden, Cholesterol and vitamin D content of eggs in the U.S. retail market, J. Food Comp. Anal. 29 (2003) 110–116.*
[b] *C.L. Taylor, K.Y. Patterson, J.M. Roseland, S.A. Wise, J.M. Merkel, P.R. Pehrsson, E.A. Yetley, Including food 25-hydroxyvitamin D in intake estimates may reduce the discrepancy between dietary and serum measures of vitamin D status, J. Nutr. 144 (2014) 654–659.*
[c] *Note: recent evidence suggests the vitamin D in this bread may not be bioavailable: S.T. Itkonen, E. Skaffari, P. Saaristo, E.M. Saarnio, M. Erkkola, J. Jakobsen, K.D. Cashman, C. Lamberg-Allardt, Effects of vitamin D2-fortified bread v. supplementation with vitamin D2 or D3 on serum 25-hydroxyvitamin D metabolites: an 8-week randomized, controlled trial in young adult Finnish women, Br. J. Nutr. 115 (2016) 1232–1239. Recent studies using vitamin D_3 in the standard fortification of bread showed excellent bioavailability and improved 25(OH)D status: B. Nikooy, T.R. Neyestani, M. Zahedirad, M. Mohammadi, S.H. Hosseini, Z. Albollahi, F. Salehi, J.M. Razaz, N. Shariatzadeh, A. Kalayi, N. Lotfollahi, M.-R. Maleki, Vitamin D-fortified bread is as effective as supplement in improving vitamin D status: a randomized clinical trial, J. Clin. Endocrinol. Metab. 101 (2016) 2511–2519. Unlike the bioaddition of light irradiated yeast in bread, vitamin D_2 bioavailability from light-exposed mushrooms is bioavailable.*
[d] *Z. Lu, T.C. Chen, A. Zhang, K.S. Persons, N. Kohn, R. Berkowitz, S. Martinello, M.F. Holick, An evaluation of the vitamin D3 content in fish: is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? J. Steroid Biochem. Mol. Biol. 103 (2007) 642–644; K.D. Cashman, K.M. Seamans, A. J. Lucey, E. Stöcklin, P. Weber, M. Kiely, T.R. Hill, Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults, Am. J. Clin. Nutr. 95 (2012) 1350–1356.*
Source: Source unless specified: From USDA, Agricultural Research Service, National Nutrient Database. Available at <http://www.nal.usda.gov/fnic/foodcomp/search/>; M.A. Johnson, M.G. Kimlin, Vitamin D, aging, and the 2005 Dietary Guidelines for Americans, Nutr. Rev. 64 (2006) 410–421.

irradiated yeast used to make bread, however, may not be available to humans [40]. Plant foods such as mushrooms which when briefly exposed to UVB produce significant amounts of vitamin D_2 and these foods have been shown to be bioavailable [41].

B Supplement Sources of Vitamin D

Supplements provide another source of intake. Vitamin D (i.e., cholecalciferol or ergocalciferol) is usually (but not always) found in multivitamin preparations at 400–1000 IU

TABLE 43.3 Vitamin D and Related Compounds Found in Some Commonly Used Vitamin D Medications in the United States

Brand Name or Type of Product	Related Compound	Dosage Forms	
Vitamin D (Supplemental)[a]			
Multivitamin	Cholecalciferol	Tablet	400−1000 IU
Calcium supplement with vitamin D	Cholecalciferol	Tablet	200−400 IU per 500−600 mg calcium
Vitamin D supplement	Cholecalciferol	Tablet, drops	400, 1000, 2000, 5000 IU
Vitamin D (Therapeutic)[b]			
Many brands	Ergocalciferol	Capsule	50,000 IU
Vitamin D in Other Medications			
Fosamax plus D	Cholecalciferol	Tablet	2800 IU weekly 5600 IU weekly
1,25-Dihydroxyvitamin D (Calcitriol)[b]			
Many brands	Calcitriol	Capsule Solution	0.25 and 0.5 μg 1 μg/mL

[a]*Available over-the-counter in North America.*
[b]*Requires a prescription from a physician in the United States.*
Source: From http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Search_Drug_Name. Accessed April 5, 2016.

per tablet. In addition to multivitamins, there are single vitamin D supplements largely available as cholecalciferol in 400 IU, 1000 IU, 2000 IU, and 5000 IU dosages. Some calcium supplements contain various amounts of cholecalciferol or ergocalciferol, in the range 200−400 IU. These supplements are intended mainly for maintenance of status, and not for repletion of vitamin D deficiency. For that purpose, higher dosage forms are available through prescription, denoted in Table 43.3 as therapeutic preparations. Not shown are cod and other fish liver oil capsules; these are available over-the-counter but their use is discouraged due to high levels of preformed vitamin A (retinol) relative to levels of vitamin D [42]. Vitamin D metabolites (primarily the active form 1,25-dihydroxyvitamin D) are available to treat clinical conditions, and these are also listed in Table 43.3.

The biological equivalency of the two forms of vitamin D, ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) in humans, has been challenged [14,17]. Both forms of dietary vitamin D are converted to their corresponding 25(OH)D form equally well. There is more rapid disappearance of $25(OH)D_2$ than $25(OH)D_3$, thus the former form of vitamin D is less effective in maintaining plasma 25(OH)D levels. However, this property makes ergocalciferol a safer form of the vitamin to use in higher doses on a daily basis. Evidence of the safe use of vitamin D_2 in postmenopausal women has been reported [43]. Reports of toxicity of vitamin D_3 supplement use have been attributed to mistakes in the formulation and manufacturing of the dietary supplements that resulted in extreme intakes exceeding 50,000 IU daily [44]. To date, normal daily use of vitamin D_3 supplements within the DRI guidelines has not resulted in vitamin D intoxication.

C Dietary Intake of Vitamin D in the American Population

Vitamin D intakes of Americans became available with analysis of NHANES III data, and subsequent surveys have been used to estimate vitamin D intakes. Estimated daily mean intakes of vitamin D from food and supplements over age and gender groups from NHANES 2011−12 [45] are shown in Table 43.4. Data shown are mean intake from food, and mean intake from food and supplements. For all age/sex groups, intake from food ranged from 148 to 264 IU [45] and studies have shown that most of food-derived vitamin D in the United States is from fortified foods [38]. There were no apparent differences in intake among racial-ethnic groups. Supplement use contributed to vitamin D intake in those using them, adding on average more than 1000 IU for supplement users. The highest use was in women over the age of 60 years. When considering total intakes of non-supplement users, the mean intake of each age group did not meet the current DRI recommendation in any age group. As many individuals do not consume supplements, it is clear from these data [45] that the current vitamin D

TABLE 43.4 Estimated Mean Usual Daily Intake of Vitamin D from Food and from Food and Supplements, NHANES 2011–12

Sex	Age (Years)	Intake from Food (IU)	Intake from Food by Supplement Users (IU)	Intake from Supplements (IU)
Male and female	2–5	264	252	344
Male and female	6–11	240	240	424
Male and female	12–19	228	256	608
Male	20–39	208	304	824
Male	40–59	228	260	1412
Male	≥60	216	200	1292
Female	20–39	156	164	1080
Female	40–59	148	140	1392
Female	≥60	176	172	1980
All	≥2	200	232	1272

Source: From What We Eat in America, NHANES 2011–2012, Day 1 food and supplements intake data. Available at: <www.ars.usda.gov/nea/bhnrc/fsrg>.

content of the U.S. food supply does not provide enough vitamin D for most Americans to meet the 2011 RDAs of 600 IU (800 IU for adults over 70 years) [4].

For those individuals with inadequate sun exposure, fortification is thought to be the most effective way to increase vitamin D intakes of the population. Many believe that mandatory fortification of commonly consumed food staples would ensure an even distribution of intakes compared to the current system of discretionary fortification of a variety of food categories. Table 43.5 shows the comparison of two countries, one with mandatory fortification (Canada) and one with only a small number of food products with discretionary fortification (United Kingdom). Both countries share similar latitudes, similar dietary habits and distribution of people of European and non-European ancestry. In Canada, milk and margarine are required by law to be fortified, and this has produced an intake of close to 200 IU for most age and sex groups [46]. In contrast, the United Kingdom had no mandatory fortification at the time of the National Diet and Nutrition Survey conducted 2008–12 [47]. Table 43.5 shows that the vitamin D intakes in the United Kingdom are half that of Canadians. The impact of mandatory fortification is also evident in the lower prevalence of Canadians with 25(OH)D levels in the "deficiency" range (9.1%) [48] compared to the United Kingdom where prevalence of vitamin D deficiency is 24% [47].

TABLE 43.5 Effect of Mandatory Food Fortification on Intakes from Food and Vitamin D Status: Canada Versus United Kingdom

Subject	United Kingdom		Canada	
Age group	No fortification		Mandatory fortification	
	Male	Female	Male	Female
Children	80 IU	76 IU	248 IU	
Adolescents	96 IU	76 IU	292 IU	216 IU
Adults	124 IU	104 IU	232 IU	204 IU
Older adults	156 IU	116 IU	268 IU	244 IU
Percent below 30 nmol/L				
Total population	24%		7.4%	

Sources: From United Kingdom survey NDNS. Available at <https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-program-for-2008-and-2009-to-2011-and-2012>; Canada: H. Vatanparast, T.J. Green, M.S. Calvo, S.J. Whiting, Despite mandatory fortification of staple foods, vitamin D intakes of Canadian children and adults are inadequate, J. Steroid Biochem. Mol. Biol. 121 (2010) 301–303; K. Sarafin, R. Durazo-Arvizu, L. Tian, K.W. Phinney, S. Tai, J.E. Camara, J. Merkel, E. Green, C.T. Sempos, S.P. Brooks, Standardizing 25-hydroxyvitamin D values from the Canadian Health Measures Survey, Am. J. Clin. Nutr. 102 (2015) 1044–1050.

D Sun Exposure as a Source of Vitamin D

Sunlight contributes UVB and UVA radiation, but only UVB permits cholecalciferol synthesis [49]. UVB includes the wavelength range of 280–315 nm, conversion of 7-dehydrocholsterol to provitamin D_3 is optimal at 290–315 nm [5]. Exposure to sunlight can be quantified in erythemal doses, that is, the appearance of reddening of the skin. A minimal erythemal dose (1 MED) causes reddening, and further exposure results in more severe sun burning. Tanning (the induction of the synthesis of the pigment melanin in the skin) also occurs but takes longer to manifest and occurs with UVA as well as UVB exposure [50].

Many environmental factors affect synthesis of cholecalciferol, as listed in Table 43.6. One that impacts a large portion of the global population is latitude. The United States provides a good example of the influence of latitude as it stretches from 20°N (Puerto Rico) to over 70°N (Alaska). It is important to recognize how latitude can impact vitamin D status. As illustrated in Fig. 43.2, there is a seasonal variation in circulating 25(OH)D at latitudes close to 50°N [15] where presumably casual exposure to sun in the late spring, summer, and early autumn has resulted in serum 25(OH)D that, on average, approaches 75 nmol/L. This is the level recommended by many researchers [6], as well as the Endocrine Society [51]. Fig. 43.2 illustrates the dramatic fall of serum 25(OH)D in winter to nearly half the concentration experienced in the spring and summer [15], presumably because dietary intake was not sufficient to provide enough vitamin D in the absence of UVB.

Studies have shown that one full body (i.e., almost completely naked) MED (1 MED) will synthesize as much as 20,000 IU of vitamin D_3 [49]. This intensity of sun exposure is not recommended due to concerns about skin phototoxicity. However, an exposure less than 1 MED will provide maximal cholecalciferol production [50]. Accordingly, one can calculate that an exposure of one-fourth of an MED to 25% of body surface is sufficient for vitamin D_3 production of 1000 IU [7]. To achieve 25% of body surface, one needs more than hands and face exposed, but also exposure of arms and legs; truncal exposure is also recommended [51]. Indeed, the "rule of nines" calculation for body surface area [52] indicates that to achieve exposure of 25% of body surface area, one would expose both lower arms (9%) and lower legs (18%). Exposure of the head could contribute to an additional 9% of surface area. The time to achieve this exposure will vary greatly by season, latitude, skin pigmentation, and use of sun-blocking lotions or cosmetics.

One can measure skin type and the resulting amount of melanin that is produced in response to UV exposure. The Fitzpatrick skin type (also called skin phototypes [50,52]) was originally developed in the United States in 1975 to facilitate UV dosage for psoriasis photochemotherapy in subjects with "white" skin, and characterized for skin types (I through IV); it was later expanded to categories V and VI, as shown in Table 43.7 [53]. These skin types vary in ability to burn and tan. The time to burn (i.e., 1 MED) reflects melanin production, and is an approximate indicator of the relative dose of UVB needed to synthesize equivalent amount of previtamin D_3 [54]. As shown in Table 43.7, skin type I needs only 40% of the time for 1 MED compared to skin type III, and skin type VI needs four times the exposure time of skin type III. The times given for ¼ MED are estimates based on exposure at 42°N (i.e., Boston), at noon, in summer, and

TABLE 43.6 Reasons for Low Sun (UVB) Exposure and Inability to Synthesize Cholecalciferol

Factor	Notes
Angle of sun in winter (November to March, inclusive at latitude 45°) not sufficient UVB	No synthesis
Being indoors and/or behind glass windows	No synthesis
Clothing, especially head-to-toe for cultural or environmental reasons	No synthesis if fabric blocks UVB. Some cloth is loosely woven and does permit UVB to penetrate
Darkly pigmented skin	More sun exposure time is needed than person with little pigment
Impairment of skin synthesis of vitamin D with age	More sun exposure time needed compared to younger person; amount made limited by substrate availability
Sunscreen use (SPF 8 or greater)	Sun protection factor (SPF) indicates amount of UV blocked: SPF blocks at 1/SPF. For example, SPF of 8 would allow 1/8 (~12%) of UV to penetrate

Sources: From B.W. Hollis, Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D, J. Nutr. 135 (2005) 317–322; M.F. Holick, Resurrection of vitamin D deficiency and rickets, J. Clin. Invest. 116 (2006) 2062–2072; M.F. Holick, Vitamin D deficiency, N. Engl. J Med. 357 (2007) 266–281.

TABLE 43.7 Categorization of Skin Type Using a Traditional Dermatological System (Fitzpatrick Skin Type) and Association of These Categories with Vitamin D Synthetic Capacity of Skin

Fitzpatrick Skin Type	Skin Color	Common Geographic Origins	Skin Response to Sun Exposure[a]	Relative MED[b]	Approximate Time for ¼ MED[c]
I	White; (blue eyes, freckled, albino)	Northern European	Always burn, never tan	0.38	4 min
II	White (blond hair, blue or green eyes)	Northern European	Burn slightly, then tan slightly	0.75	6 min
III	White (brown eyes, darker complexion)	Southern European, Middle East	Rarely burn, tan moderately	1.0	7 min
IV	White ("Mediterranean")		Never burn, tan darkly	1.3	10 min
V	Brown	Asian, Native American, Pacific Islander	Never burn, tan darkly; Oriental or Hispanic skin	2.0	13 min
VI	Black	African	Never burn, tan darkly; Black skin	3.8	21 min

[a]*Characteristics of previously unexposed skin after 30 min direct exposure to sun.*
[b]*Relative MED dose of UVB exposure.*
[c]*Time estimated for sun exposure at 10:30 a.m. on June 21, to reach ¼ MED at 11.5°N, 29°N, and 42.5°N (values for 62.5°N would be double) from Webb and Engelsen reference [7].*
Sources: From B.A. Gilchrest, Sun protection and vitamin D: three dimensions of obfuscation, J. Steroid Biochem. Mol. Biol. 103 (2007) 655–663; M.F. Holick, M. Jenkins, The UV Advantage, Simon & Schuster, New York, NY, 2003; T.B. Fitzpatrick, The validity and practicality of sun-reactive skin types I through VI, Arch. Dermatol. 124 (1988) 869–871.

may be used to calculate the exposure time to reach a vitamin D dose of 1000 IU when 25% of body surface is exposed [7]. Other factors related to timing, such as age, have not been considered.

Thus, there is no single answer to how much sun exposure is needed to achieve and maintain an adequate vitamin D status. Some dermatologists have advocated for no sun exposure [50]; however, the World Health Organization Report [55] on solar UV radiation indicates that it is not appropriate to strive for zero sun exposure as this would create a huge burden of skeletal disease from vitamin D deficiency. Nonetheless, it is important to avoid excess exposure since this has been linked with skin cancers and skin photoaging [52]. A rational scheme to achieve skin synthesis of cholecalciferol without significant risk of overexposure has been published [7]. While this subject remains a source of considerable controversy, the public now has access to information that will allow weighing of personal risk and benefits of sun exposure. Sensible sun exposure is most important in growing children. Estimates of how much vitamin D_3 young Americans produce from their everyday outdoor UV exposure in the north (45°N) and south (35°N) for each season were reported [56]. The resulting estimates suggest that most American children are not getting enough sun exposure to meet either minimal ($\sim$600 IU/day) or optimal ($>$2000 IU/day) vitamin D requirements [56].

IV VITAMIN D NUTRITIONAL STATUS ASSESSMENT AND RELATION TO DISEASE RISK

A Indicators of Vitamin D Status

1 The Main Indicator of Vitamin D Status Is 25-Hydroxyvitamin D

Vitamin D status is evaluated by measuring the circulating levels of 25(OH)D, the level of which is related to the combined contributions of diet (including supplements) and skin synthesis. Measuring cholecalciferol and ergocalciferol is technically difficult and will only provide information on recent exposure (within the past 72 hours or less) [49]. In contrast, cholecalciferol and ergocalciferol are stored in fatty tissues (adipose, liver, muscle), and possibly can be made available for conversion to 25(OH)D when needed. However, these stores cannot maintain 25(OH)D levels through a long period of dietary or UVB deprivation [13]. There is some uncertainty as to whether storage of cholecalciferol in adipose is available to the body. Studies show that in obese individuals, serum levels of cholecalciferol, ergocalciferol, and 25(OH)D are lower than in nonobese individuals [57,58]. This may relate to cholecalciferol and ergocalciferol being sequestered in adipose without an ability to be released.

Measurement of 25(OH)D provides the best assessment of vitamin D "stores," that is, the form of vitamin D available to tissues for synthesis of 1,25-dihydroxyvitamin D by endocrine or paracrine/autocrine pathways (Fig. 43.1). This is because 25(OH)D, although not the active form of vitamin D, rises with intake and/or sun exposure, and declines with combined sun avoidance and low intake. The measurement units are nmol/L or ng/mL, and the conversion factor between these different units is the following: 1 ng/mL = 2.5 nmol/L. Measurement of 1,25-dihydroxyvitamin D, the active vitamin D metabolite, is not the preferred indicator (biomarker) of vitamin D status but rather a reflection, primarily, of the need for calcium and phosphate [5], as described earlier. Further, the amount of 1,25-dihydroxyvitamin D, in blood is influenced by renal function and other dietary and hormonal factors.

2 Classification of Vitamin D Status by Serum 25(OH)D

Until about 10 years ago [6] there was a lack of understanding that tissues needed a steady supply of 25(OH)D as a precursor for cellular synthesis of 1,25(OH)2D that functioned in the paracrine/autocrine pathways. Moreover, there was a lack of appreciation that vitamin D deficiency (low serum levels of 25(OH)D) may contribute to the risk of developing many chronic conditions beyond rickets and osteomalacia. For the longest time, the cut-off serum concentration determining vitamin D deficiency has been serum 25(OH)D below 25 or 30 nmol/L, as below this value, the patient has or will soon experience rickets (in children) or osteomalacia (in adults) [49]. New data over the past decade indicates other disease states may be affected but generally when 25(OH)D is much higher than that needed to prevent rickets and osteomalacia [51].

There is a clear disagreement as to what the normal range of 25(OH)D should be to reflect adequate vitamin D status. Table 43.8 outlines the various cut-off points that have been used in the two most recent guidelines for vitamin D [4,51], highlighting disagreement in the definition of "deficient" and "insufficient." In the 2011 DRI report on setting (for the first time) Estimated Average requirement (EAR), and RDA levels, as well as revising Tolerable Upper-Intake Level (UL) values for vitamin D [4], the level of 25(OH)D that represented adequacy for almost everyone (i.e., the RDA) was defined as a serum level of 25(OH)D at or above 50 nmol/L, as this level was deemed to provide for maximal calcium absorption, minimal risk of rickets in children, reduced fracture risk in adults, and minimal risk of osteomalacia in adults. The level of 30 nmol/L was set as the cutoff for deficiency. The level of 40 nmol/L was set as the EAR which should represent sufficiency for 50% of the population. In strong opposition to this cut-off value for sufficiency, the Endocrine Society has set sufficiency at 75 nmol/L based on suppression of PTH levels which reflects the amount required for calcium absorption and they defined "deficiency" at <50 nmol/L [51].

3 Issues Related to 25-Hydroxyvitamin D Measurement

There are many methods for determining circulating levels of 25(OH)D. A radioimmunoassay (RIA) and a competitive protein binding assay have been employed clinically, as well as an enzyme immunoassay [59].

TABLE 43.8 Classification of Vitamin D Status by Serum 25(OH)D[a] and Disease Risk

Serum 25(OH)D	Category
<20 nmol/L	Vitamin D deficiency
<30 nmol/L	At risk for rickets or osteomalacia
<50 nmol/L	Not at risk for clinical rickets or osteomalacia
30–75 nmol/L	Vitamin D insufficiency defined for at-risk populations with chronic conditions
≥75 nmol/L	Vitamin D adequacy for at-risk populations
125–150 nmol/L	Level set by IOM as UL
250 nmol/L	Level of 25(OH)D when 10,000 IU is ingested chronically, and level where no adverse effects are detected. Potential for adverse effects above this level

[a]*25-hydroxyvitamin D; to convert nmol/L to ng/mL, divide by 2.5.*
Sources: From Institute of Medicine, Dietary Reference Intakes for Calcium Phosphorus, Magnesium, Vitamin D and Fluoride, National Academy Press, Washington, DC, 2011; M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M.H. Murad, C.M. Weaver, Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline, J. Clin. Endocrinol. Metab. 96 (2011) 1911–1930.

Combination Liquid Chromatography-Mass Spectrometry (LC-MS) is now being used for national survey data, and older data are being "adjusted" to this standard methodology to allow for comparison between surveys. The NHANES data shown below were analyzed using LC-MS as were data in Table 43.5. All of the widely used clinical laboratory methods perform reasonably well in identifying clinically important low serum 25(OH)D. It is important to mention that all clinical laboratories performing 25(OH)D testing should take part in external quality assessment programs; particularly because some of the commercially available assays may fail to recognize and accurately measure $25(OH)D_2$. Measurement of blood 25(OH)D is best performed on fasting serum sample as lipid-rich plasma can interfere with the assay so high-fat meals should be avoided at least 12 hours before the blood draw. Otherwise, blood may be taken at any time of day since there is no observed pronounced circadian variation in serum 25(OH)D. As previously indicated, results are reported as nmol/L or ng/mL. The conversion factor between these different units is the following: 1 ng/mL = 2.5 nmol/L.

A "new" metabolite of vitamin D was first detected in the blood of newborns and initially was measured as 25(OH)D [60]. This molecule, 3-epi-$25(OH)D_3$ arises in vivo from ingested cholecalciferol but is not a precursor of the active form (calcitriol) and thus does not have "vitamin D activity." In adults it represents about 5% of 25(OH)D levels [58]; however, in infants it can be much higher, and therefore overestimate vitamin D status [60].

When repleting deficient patients, one expects the serum 25(OH)D response to high dose supplementation with cholecalciferol or ergocalciferol to reach a plateau after 3 months of continuous treatment [15]. Therefore, if a patient's response to vitamin D supplements is to be monitored by taking repeated blood measures over time, samples should be checked no sooner than 3 months after treatment initiation. Monitoring of routine supplementation use is not necessary, but in situations where severe deficiency is being treated or there is reason to believe the patient may have impaired intestinal absorption, assessment of serum 25(OH)D would indicate the effectiveness of therapy.

4 Vitamin D Status of Americans: NHANES Data

The 2007–10 NHANES survey data provide current vitamin D status of Americans [58]. Mean levels of 25(OH)D are shown in Table 43.9. As well, the prevalence below

TABLE 43.9 Mean Serum Levels of 25(OH)D[a] and Prevalence of Vitamin D Deficiency (<30 nmol/L), of Not Meeting EAR (<40 nmol/L), of Insufficiency (< 50 nmol/L), of Less Than Optimal (<75 nmol/L), and of Exceeding UL (>125 nmol/L), NHANES 2007–10

Group	Age Group (Years)	nmol/L	% <30 nmol/L	% <40 nmol/L	% <50 nmol/L	% <75 nmol/L	% >125 nmol/L
All	≥1	68.0	5.9	13	24	64	2.3
Hispanic	≥1	57.2	6.4	18	36	83	0.1
NHB	≥1	46.6	24	43	62	91	0.4
NHW	≥1	75.2	2.3	5.8	13	53	3.4
Female	≥1	69.1	6.9	23	41	61	3.6
Male	≥1	66.8	4.9	14	31	66	1.0
M&F	1–5	76.5	0.7[b]	3.0[b]	9.7[b]	56[b]	1.2[b]
M&F	6–11	72.2					
M&F	12–19	65.8	5.9	14	25	70	2.3
M&F	20–39	64.6	8.2	18	30	69	2.9
M&F	40–59	67.9	5.9	13	24	64	2.1
M&F	≥60	71.0	5.7	13	22	56	2.6

[a] *25-hydroxyvitamin D. To convert nmol/L to ng/ml, divide by 2.5.*
[b] *Values are for ages 1–8 years.*
NHB, non-Hispanic Black; NHW, non-Hispanic White; EAR, Estimated Average Requirement; UL, Upper Level; M&F, male and female.
Source: From L.R. Schleicher, M.R. Sternberg, A.C. Looker, E.A. Yetley, D.A. Lacher, C.T. Sempos, C.L. Taylor, R.A. Durazo-Arvizu, K.L. Maw, M. Chaudhary-Webb, C.L. Johnson, C.M. Pfeiffer, National estimates of serum total 25-hydroxyvitamin D and metabolite concentrations measured by liquid chromatography–tandem mass spectrometry in the US population during 2007–2010, J. Nutr. 146 (2016) 1051–1061.

the various cutoffs for 25(OH)D levels are given: percent below 30 nmol/L defines risk of vitamin D deficiency; percent below 40 nmol/L is prevalence below EAR; percent below 50 nmol/L is prevalence of insufficiency according to Institute of Medicine (IOM) [4]; percent below 75 nmol/L is less than optimal ("deficiency" according to the Endocrine Society) [51]; and percent above 125 nmol/L is prevalence above the UL. In Table 43.9, for the total population at or over the age of 1 year, three-quarters were sufficient (i.e., >50 nmol/L), and a small percentage (~6%) was deficient (at-risk for rickets and osteoporosis). More importantly, these prevalences were not distributed equally across racial/ethnic groups. Non-Hispanic Blacks show high risk for deficiency which is reflected in prevalence of rickets in this group (see Section IV.B.1, Nutritional Rickets). Children ages 1−11 years showed the least insufficiency. There were few differences between the other age groups. Interestingly, older adults were no worse off than younger adults, as supplement use in older adults was higher [58].

As these NHANES data were collected in the northern states during sunny seasons and the southern states during winter months, the 25(OH)D data represent optimal levels of sun exposure opportunities for participants. Even so, winter season showed a 12 nmol/L lower 25(OH)D level; and latitude also a significant factor, with "northern" being 10 nmol/L lower than "southern." Other significant predictors of serum 25(OH)D concentration were: dietary intake of vitamin D (<200 IU vs ≥200 IU); supplement intake (<400 IU vs ≥400 IU); infection/inflammation (C-reactive protein <10 mg/L vs ≥10 mg/L); and body weight (higher vs lower BMI). Self-reported sun protection behavior did not show any significance.

5 Predicting 25(OH)D Levels

Serum 25(OH)D is influenced by exposure of skin to UVB and by dietary intakes of ergocalciferol or cholecalciferol. From its use in many research trials as well as in dosing studies [15], it can be estimated that 40 IU (1 μg) of cholecalciferol daily raises serum 25(OH)D by 2 nmol/L at basal levels, that is, in persons having serum 25(OH)D less than 50 nmol/L [61]. Once past this level, 40 IU (1 μg) will raise 25(OH)D by 1 nmol/L [62]. Sun exposure, as described earlier, will also impact on serum levels of 25(OH)D. Effects of dietary vitamin D and skin synthesis of cholecalciferol are additive; both contribute to serum total 25(OH)D [13]. Thus, it should be possible to predict vitamin D status in light-skinned individuals. For example, if a background of moderate sun exposure during the summer achieves a serum 25(OH)D of 50 nmol/L, then a further 1000 IU (25 μg) per day of dietary cholecalciferol would be needed to reach a serum 25 (OH)D level of 75 nmol/L.

Most of the studies determining dietary or solar contributions to circulating 25(OH)D have been conducted in light-skinned subjects. Our current understanding of dietary intakes needed to raise serum 25(OH)D is lacking for African Americans and others with darker skin, but evidence points to significant differences that should be taken into consideration when setting dietary requirements.

6 Other Biomarkers of Vitamin Deficiency

While it is well established that serum 25(OH)D is the best static indicator of vitamin D status, functional indicators provide information on how the deficiency/insufficiency is impacting on the health of the person. They also confirm that a deficiency exists, and may confer additional information about the need to monitor the efficacy of corrective interventions. The most common responsive functional indicator examined to date is the suppression of secondary hyperparathyroidism, based on the endocrine function of 1,25-dihydroxyvitamin D in providing enough calcium through active intestinal absorption to suppress PTH levels that were raised with hypocalcemia [63]. Performance indicators such as the "get up and go" test vary in proportion to serum 25(OH)D status; they reflect muscle strength and balance that are negatively affected in vitamin D insufficiency [64]. Pain is also associated with vitamin D deficiency, and minimal trauma fractures may also occur. These alone are not specific enough for diagnosis; however, they should be triggers to seek vitamin D testing.

B Clinical Conditions Associated With Vitamin D Deficiency and Insufficiency

1 Nutritional Rickets

Vitamin D deficiency results in rickets and osteomalacia, the former name used to describe the clinical signs observed in children, while the latter name being those signs in adults. Nutritional rickets is defined as "a disorder of defective chondrocyte as differentiation and mineralization of the growth plate and defective osteoid mineralization" [65]. It is caused by vitamin D deficiency and/or low calcium intake in children. Its diagnosis is made based on history, physical examination, and biochemical testing, to be confirmed by radiographs [65]. As outlined in Table 43.10, the physical signs of rickets in children present as severe bone, muscle, respiratory, and overall growth problems. Rickets is a disabling disease that usually begins in early childhood, before the age of 18 months [5]. Once the child can stand, gravity worsens the bone changes so that the classic picture of inward or outward bowed legs is observed. In addition to rickets occurring in very young children, a second peak can occur between 5 and 15 years of age [66]. Once the

TABLE 43.10 Physical and Clinical Signs of Rickets

Body System Affected	Physical and Clinical Signs
Bone	• poor mineralization
	• hypertrophy of costochondral junctions
	• rachitic rosary: involution of the ribs and protrusion of the sternum
	• tibial and femoral bowing (inward or outward)
	• chest deformation
	• delayed closing of fontanels
	• bone pain[a]
Teeth	• delayed eruption
	• enamel hypoplasia
	• early dental caries
Muscle	• delayed motor development
	• toneless and flabby legs
	• waddling gait[a]
Heart	• myocardiopathy
Lungs	• defective ventilation
	• respiratory obstruction
	• infections
Other	• secondary hyperparathyroidism
	• low plasma phosphate
	• tetany and seizures
	• hypochromic anemia
	• fatigue[a]

[a]*Symptoms of osteomalacia.*

Source: From M.E. Holick, Resurrection of vitamin D deficiency and rickets, J. Clin. Invest. 116 (2006) 2062–2072.

epiphysis has closed, the condition is called osteomalacia. In adolescent cases of rickets characterized in Denmark [68], the authors identified 112 patients with nutritional rickets (due to low vitamin D) of whom 74% were immigrants to Denmark.

Reporting of rickets is not required in most countries, therefore prevalence is difficult to estimate. However, "reemergence" of nutritional rickets was noticed in the United States in the 1990s. Weisberg et al. summarized 22 case reports of rickets in the United States published from 1986 to 2003 [67]. These cases were predominately in African-American children ages 4–54 months, of whom 96% were breast-fed. In Canada, a survey of pediatricians over 2 years found that there had been 104 cases of infant rickets primarily those living in northern Canada, 94% of whom were breast-fed with no vitamin D supplementation [68]. Studies such as these have alerted authorities such as the IOM [4] and the American Academy of Pediatrics [69] to the need for vitamin D supplementation in breast-fed infants as well as in infants not consuming sufficient fortified formula to supply 400 IU per day [69]. In 2016, global consensus recommendations on preventing rickets were published [65] that also endorse vitamin D supplementation of breast-fed infants at the level of 400 IU per day. Alternatively, some researchers have found that supplementing mothers throughout pregnancy [70] or through lactation [71] can result in breast milk having adequate vitamin D activity (as cholecalciferol and 25(OH)D) to meet the needs of the infant. The amount needed by lactating mothers, however, to give the equivalency of 400 IU per day to the infant is not known but has been estimated to be as high as 6400 IU [71].

The musculoskeletal effects of rickets arise primarily because of a lack of calcium and phosphate for bone mineralization and muscle function. With chronic severe vitamin D deficiency, there is malabsorption of calcium and phosphate with a resultant hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism. These lead to impaired growth plate development and bone mineralization [5]. Not all cases of rickets arise from a primary deficiency of cholecalciferol (or ergocalciferol). Although rare, there are many other types of rickets, as shown in Table 43.11, many of these rarer types are inherited forms of rickets due to mutations in important receptors (e.g., VDR) or enzymes (e.g., 1-hydroxylase) involved in vitamin D metabolism or action. Some forms of rickets are not due to problems with vitamin D, for example, calcium deficiency rickets, which is common in African countries.

2 Osteomalacia

Osteomalacia is characterized by muscle atrophy (i.e., a waddling gait), by bone pain, and by fatigue; these signs are also seen in rickets [5]. There are drugs which cause osteomalacia. The most common drug-induced osteomalacia is due to anticonvulsant therapy, particularly phenytoin, barbiturate derivatives, and carbamazepine. Although the exact mechanisms require further study, it is thought that these drugs result in greater inactivation of 1,25-dihydroxyvitamin D, as well as reducing 25(OH)D, by inducing the renal enzyme 24-hydroxylase [32].

The diagnosis of osteomalacia is often missed, even when bone pain and muscle weakness are present [72]. An examination using iliac crest biopsies at autopsy from otherwise healthy male and female individuals (primarily accident victims) were quantified for indices of osteomalacia and related to serum 25(OH)D [73]. In subjects

TABLE 43.11 Types and Treatment of Rickets

Type of Rickets	Metabolic Abnormality Mechanism	Treatment
Vitamin D dependent	Insufficient cholecalciferol production from UVB exposure and lack of dietary cholecalciferol or ergocalciferol	Supplemental cholecalciferol or ergocalciferol or sun exposure (with appropriate UVB); aggressive therapy needed initially
Calcium-deficiency rickets	Low calcium intake	Provide adequate calcium and cholecalciferol or ergocalciferol
Fat malabsorption (e.g., cystic fibrosis)	Insufficient cholecalciferol production from UVB exposure and lack of dietary cholecalciferol or ergocalciferol	Subcutaneous or intramuscular injections of cholecalciferol recommended
Hereditary vitamin D-dependent rickets Type 1	Inactive or absent renal 1-hydroxylase enzyme	Provide 1,25-dihydroxyvitamin D (or 1-hydroxyvitamin D)
Hereditary vitamin D-dependent rickets Type 2	Mutations in VDR prevent normal actions of 1,25-dihydroxyvitamin D	Respond to high doses of either cholecalciferol or 1,25-dihydroxyvitamin D
Hereditary vitamin D-dependent rickets Type 3	Abnormal hormone response element binding protein	Respond to high doses of either cholecalciferol or 1,25-dihydroxyvitamin D
Hypophosphatemic rickets	Phosphatemia (acquired and inherited) and decreased 1-hydroxylase enzyme	Intravenous phosphate; remove tumor
Tumor-induced osteomalacia	Tumor secretes phosphate factor that causes phosphatemia (acquired and inherited) and decreased 1-hydroxylase enzyme	

Source: From M.E. Holick, Resurrection of vitamin D deficiency and rickets, J. Clin. Invest. 116 (2006) 2062–2072.

where 25(OH)D concentrations were below 75 nmol/L, over one-third experienced mineralization defects, that is, pathological increase in osteoid, while no defects were observed in individuals with serum 25(OH)D exceeding 75 nmol/L. Osteomalacia may be misdiagnosed as osteoporosis, but a rise in serum alkaline phosphatase is seen only in the former condition [72]. Osteomalacia in younger women when the pelvis is severely malformed can cause cranio-pelvic disproportion, obstructed labor, and need for Cesarean section in some parts of the world [74].

3 Osteoporosis

The "classic" vitamin D deficiency diseases are rickets and osteomalacia; however, it is now evident that some chronic diseases appear to have a sun-exposure etiology. In the early 2000s, it was recognized that other health outcomes were affected by vitamin D status [6]. A long list of diseases and health disorders has been under investigation ever since, including osteoporosis (Table 43.12). The mild, secondary hyperparathyroidism that occurs with vitamin D insufficiency (i.e., levels of 25(OH)D below 75 nmol/L) may cause increased bone turnover and bone loss—a clinical picture compatible with osteoporosis [75]. Vitamin D insufficiency is very commonly found in patients with osteoporosis and contributes to the clinical presentation of osteoporosis (low bone density, fractures, falls), as well as the variety of conditions that have been found to have a higher incidence when associated with insufficient vitamin D. Thus vitamin D insufficiency or deficiency should be considered in any patient with osteoporosis. Even after osteoporosis treatment has been initiated, a recent survey of patients attending osteoporosis clinics found approximately 50% of the patients had suboptimal serum 25(OH)D levels. Vitamin D insufficiency or deficiency should always be considered in patients with osteoporosis who do not appear to be responding to therapy (i.e., continuing to lose bone density, or continuing to suffer fragility fractures) [75].

Several pathological conditions contribute to secondary vitamin D deficiency that may lead to osteoporosis or osteomalacia. These include gastrointestinal disease, kidney disease, and drug-induced deficiency. Examples of the latter are shown in Table 43.13. Several common gastrointestinal disorders result in malabsorption [51], resulting in incomplete or absence of absorption of cholecalciferol/ergocalciferol. These include celiac disease, inflammatory bowel disease, gastrectomy, pancreatic insufficiency, and cystic fibrosis. As well, vitamin D deficiency can be precipitated from gastrointestinal

TABLE 43.12 Health Outcomes Associated with Vitamin D

Skeletal Diseases
• Rickets
• Osteomalacia
• Osteoporosis
Cancer
• Breast
• Prostate
• Colon
• Ovarian
Autoimmune Diseases
• Multiple sclerosis
• Diabetes (Type 1)
• Irritable bowel disease
• Rheumatoid arthritis
Adverse Pregnancy Outcomes
• Preeclampsia
• Gestational diabetes mellitus
• Preterm birth/small gestational age
Cardiovascular Disease
• Hypertension
• Arteriosclerosis
Microbial Infections
• Tuberculosis
Renal Disease
• Chronic renal failure
• End-stage renal disease
Malabsorption Disorders
• Celiac
• Cystic fibrosis
Neuromuscular Disorders
• Parkinson's
• Multiple sclerosis
Neuronal Disorders
• Dementia/Alzheimer's
• Depression
• Autism

Sources: From M. Wacker, M.F. Holick, Vitamin D—effects on skeletal and extraskeletal health and the need for supplementation, Nutrients 5 (2013) 111–148; M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M.H. Murad, C.M. Weaver, Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline, J. Clin. Endocrinol. Metab. 96 (2011) 1911–1930; J.J. Cannell, On the etiology of autism, Acta Paediatr. 99 (2010) 1128–1130; G.A. Mozaffarian, Investigating factors of decline in cognitive function or dementia, Arch. Intern. Med. 171 (2011) 266–267; L.F. Morrone, P. Bolasco, C. Camerini, G. Cianciolo, A. Cupisti, A. Galassi, S. Mazzaferro, D. Russo, L. Russo, M. Cozzoline, Vitamin D in patients with chronic kidney disease: a position statement of the working group: "Trace Elements and Mineral Metabolism" of the Italian Society of Nephrology, J. Nephrol. 29 (2016) 305–328; E. Kwon, L.G. Gallagher, S. Searles Nielsen, G.M. Franklin, C. T. Littell, W.T. Longstreth, P.D. Swanson, H. Checkoway, Parkinson's disease and history of outdoor occupation, Parkinsonism Rel. Disord. 19 (2013) 1164–1166.

surgeries such as bariatric surgery (intestinal bypass) or small bowel resection (short gut syndrome).

Bone mineral density (BMD), believed to be the early indicator for risk of osteoporotic fracture, can be affected by vitamin D status. Randomized controlled trials (RCTs) in older adults have evaluated the effect of either vitamin D_3 or D_2 on BMD. A systematic review of the literature found that supplementation with 800 IU of vitamin D_3 daily, in combination with calcium reduced fracture risk [76]. A major limitation has been compliance rates for consuming the supplements of less than 80% and lack of assessment of vitamin D status (i.e., no 25(OH)D levels measured). Poor compliance with vitamin D supplementation was an issue noted more often in the large community-based trials [76]. Further, dose provided is an issue. When the daily vitamin D dose was only 10 μg (400 IU), there was no reduction in fracture risk [76,77]. This finding is not unexpected as that low amount of vitamin D_3 would only result in a small rise in serum 25(OH)D, approximately 7–10 nmol/L and deficient subjects (<30 nmol/L) receiving this small dose likely would not have serum 25(OH)D above 50 nmol/L.

A 2014 Cochrane review concluded that vitamin D alone was not sufficient but supplementation with both vitamin D and calcium reduced the risk of fragility fractures [78]. With respect to systematic reviews, the contribution of the Women's Health Initiative trial [79] is large, but this study gave only 400 IU to participants in addition to 1000 mg calcium, and allowed the placebo group to take supplements. In another systematic review, also published in 2014, the role of vitamin D was downplayed [80]; however, even after including studies where subjects were already sufficient (>50 nmol/L) there was a significant effect of vitamin D on femoral neck BMD. Including studies of vitamin D in participants already adequate for vitamin D with respect to bone health (>50 nmol/L) has caused confusion and as is discussed below, made the scientific community more cognizant of the difficulties in interpreting RCTs with vitamin D, as is discussed in Section VII.

TABLE 43.13 Medications Altering Vitamin D Status

Mechanism of Action	Condition for Use: Drug Family
Drugs reducing vitamin D absorption	Cholesterol-lowering agent: cholestyramine Weight loss drug orlistat and food additive olestra
Drugs reducing plasma vitamin D levels	Cholesterol lowering: statins
Drugs reducing 25(OH)D levels	Anticonvulsant medications: carbamazepine, phenobarbital, and phenytoin, gabapentin Antiretroviral agents: ritonavir and efavirenz, valproic acid Heartburn, peptic ulcer (histamine H2 receptor antagonist): cimetidine
Drugs impairing vitamin D metabolism	Oral corticosteroids: glucocorticoids Psoriasis: calcipotriol/calcipotriene

Source: From National Osteoporosis Foundation. Available at: <http://www.webmd.com/osteoporosis/features/the-truth-about-vitamin-d-drug-interactions>.

4 Muscular Health and Falls Risk

The risk of fracture in vitamin D deficiency is not only increased due to low bone density, but also to the greater risk of falls. Studies show that vitamin D reduces fracture risk in part by reducing risk of falls [81]. Here, vitamin D is acting to increase muscle strength and may thus improve balance and reduce falls. In a study of adults 65 years and older, neuromuscular performance tests such as chair stands and walking tests were performed and related to vitamin D status (Fig. 43.4). Performance improved as vitamin D status improved, up to a cutoff for serum 25(OH)D of 50−75 nmol/L. Vitamin D acts through both the endocrine and paracrine/autocrine systems to improve muscle health [81]. Atrophy of type II muscle fibers, those most responsible for muscle strength, is seen in vitamin D deficiency [81]. Even younger adults and athletes with low vitamin D status may show compromised muscle strength [82].

A study published in 2016 saw no benefit of vitamin D supplementation for falls risk, and may have detected adverse events associated with supplementation to adults 70 years and older [83]. For a year, two treatments were tested: a monthly dose of 60,000 IU of vitamin D_3 or a monthly dose of 24,000 IU vitamin D_3 together with 300 μg 25(OH)D (assumed to provide 15,000 IU), compared to a low dose group which was monthly 24,000 IU. At baseline only 58% of the subjects had serum 25(OH)D <50 nmol/L. Falls incidence was highest in both high treatment groups. These data suggest too much vitamin D may be harmful to muscle strength. A second explanation for the findings is that monthly dosing is not appropriate, especially when given in very high doses. Thus while there may be some contradictory findings for falls risk, earlier studies using daily dosing showed that preventing vitamin D insufficiency reduced risk of falls in older adults [84].

C Vitamin D Insufficiency as a Public Health Problem

Research is ongoing concerning how vitamin D status can impact on chronic disease. Both the endocrine and paracrine pathways are implicated as mechanisms for understanding how vitamin D is involved in chronic disease prevention. As shown in Fig. 43.1, the endocrine pathway, which maintains calcium and phosphate homeostasis, impact on bone, kidney, muscle, and cardiovascular health. The paracrine/autocrine pathways provide 1,25-dihydroxyvitamin D for cell functioning. Systems that are affected are those involving cell differentiation such as the immune system. These paracrine pathways may impact on a variety of diseases under investigation such as cancer, infectious, neurologic, and autoimmune diseases [81]. A summary of disease conditions under investigation as being influenced by vitamin D status are listed in Table 43.12. A brief summary of research to date is provided for some of these conditions.

1 Vitamin D and Cancer Prevention

The connection between vitamin D status and cancer becomes clearer if one looks to the disruption of normal cell activities which characterize cancers. Malignancies are characterized by disregulation in cell growth and differentiation. Observational studies of sun exposure or vitamin D status in relation to cancer risk early on showed protective relationships between sufficient vitamin D status and lower risk of cancer [81]. A Cochrane review of vitamin D supplementation for prevention of cancer in adults in 2014 found there was no strong evidence that vitamin D supplementation decreases or increases cancer occurrence in elderly community-dwelling women [85]. However, epidemiological studies of both colorectal and breast cancers show a consistent inverse association for

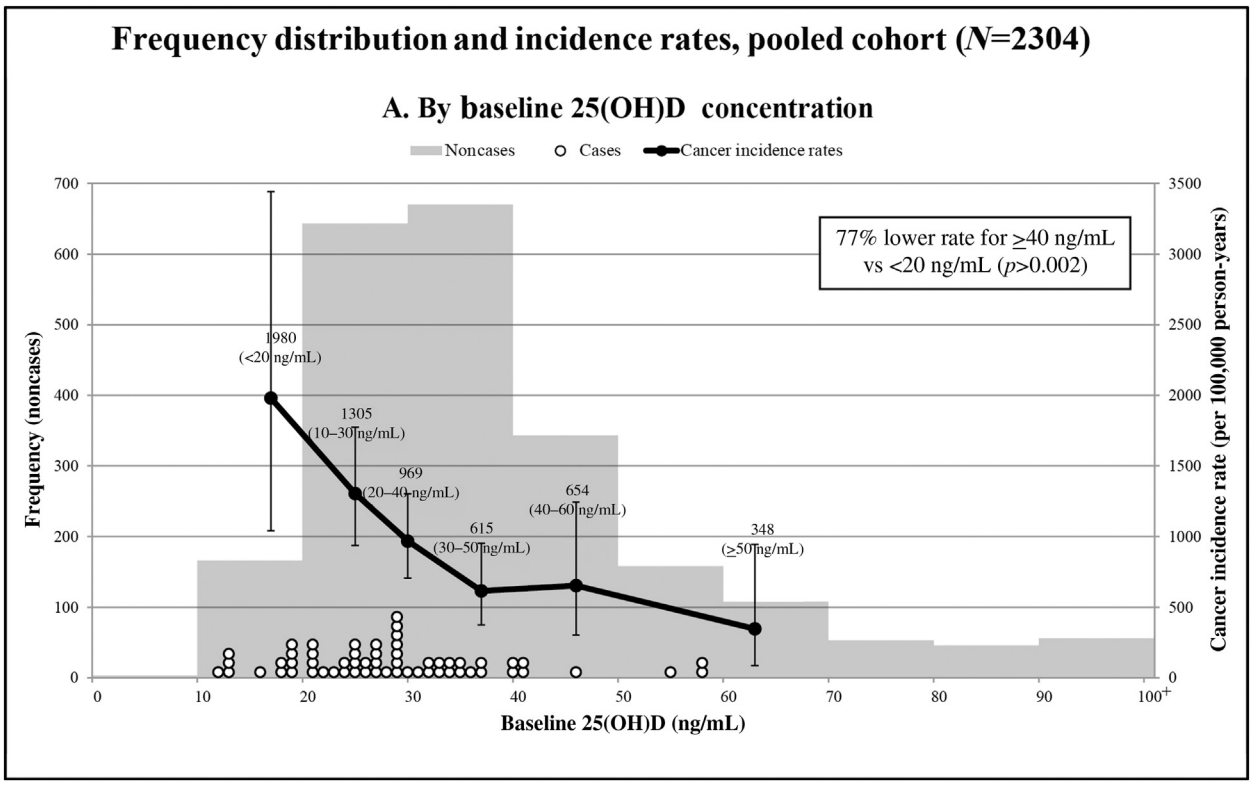

FIGURE 43.5 Frequency distribution and cancer incidence rates by 25(OH)D concentration of the pooled cohorts ($N = 2304$) followed for ~4 years. The bars represent the number of noncases and the white dots are the 25(OH)D concentration for each cancer case. *From S.L. McDonnell, C. Baggerly, C.B. French, L.L. Baggerly, C.F. Garland, E.D. Gorham, J.M. Lappe, R.P. Heaney, Serum 25-hydroxyvitamin D concentrations ≥ 40 ng/mL are associated with >65% lower cancer risk: Pooled Analysis of Randomized Trial and Prospective Cohort Study, PLoS One 11 (2016) e0152441.*

both incidence and mortality, but clinical trials have not borne out this relationship [86]. For colon and colorectal cancers, it is highly plausible vitamin D has an effect due to its roles in differentiation and gut barrier function [87]. A meta-analysis of 11 eligible studies of breast cancer and vitamin D found that a serum 25(OH)D level of 120 nmol/L was associated with a 50% lower risk of developing breast cancer [88].

Indeed, it may be that baseline levels of 25(OH)D determine how well vitamin D status is impacted in supplementation trials. Two cohorts of women 55 years and older, one being an RCT of vitamin D originally intended as an osteoporosis study, and the other a population-based prospective study, were combined to address this question [89]. Fig. 43.5 plots baseline 25(OH)D against the frequency of cancer over a 4-year period. When frequency was compared between cases <20 ng/mL (50 nmol/L) 25(OH)D and those occurring at or above 40 ng/mL (100 nmol/L), there was a 77% lower rate of all cancers, with the most common cancer being breast cancer. A similar relationship showing a 71% lower rate was seen when frequency was plotted against mean achieved 25(OH)D for all tests during the 4-year time period. Thus achieving a serum 25(OH)D of 100 nmol/L appears to be the key component to reducing incidence and may explain the lack of success in other trials.

There remains the debate as to whether vitamin D status should be improved by diet or sun exposure. In a study of persons with a diagnosis of nonmelanoma skin cancer, for whom sun exposure was the likely cause of this cancer, there were lower rates of second cancers (colon, gastric, and rectal cancers) [90]. These studies underscore the need for better understanding of the role of vitamin D, which for many people is through sun exposure. While sun exposure increases the risk of developing skin cancer [50], other evidence suggests it has the potential to reduce the risk of developing more severe forms of cancer [81].

2 Vitamin D and Immunity

Vitamin D's action in the immune system is well known, and as a consequence vitamin D has been studied with respect to its role in infectious disease as well as in autoimmune diseases [81]. Vitamin D plays a critical role in the innate immune system, which is initiated in

macrophages at first contact with an invading pathogen. Tuberculosis, influenza, and viral upper respiratory tract infections are under investigation. While consistent, strong evidence has not yet been found, there is convincing evidence that tuberculosis especially can be reduced, or severity of this condition reduced, with improvement in vitamin D status [91].

Poor vitamin D status has been shown to be associated with developing autoimmune diseases such as type 1 diabetes mellitus, multiple sclerosis, and inflammatory bowel disease [81]. Type 1 diabetes in children is related to serum 25(OH)D [92]. Even type 2 diabetes may be reduced with vitamin D supplementation [93]. Epidemiological studies have suggested that high circulating levels of vitamin D are associated with a lower risk of multiple sclerosis. Overall, studies support a protective effect of vitamin D, but there are some unanswered questions, including the mechanism of action and how genetic variations modify the effect of vitamin D status [94]. It merits mention that promising clinical evidence is emerging suggesting that vitamin D status influences the relapse rate and radiological lesions in patients with multiple sclerosis [95]. It remains unclear whether vitamin D can influence the course of multiple sclerosis progression, or influence the prevention of multiple sclerosis.

3 Vitamin D and Prevention of CKD and Cardiovascular Disease

The kidney is critically important for vitamin D metabolism, serum 25(OH)D concentrations are lower in patients with varying stages of renal function [96–98]. This relationship was characterized in the general U.S. population using NHANES III data, demonstrating significantly lower serum 25(OH)D only in those survey participants with low estimated glomerular filtration rates (eGFRs), that is, with values <29 mL/minute/1.73 m^2 [97]. These findings support the hypothesis that vitamin D status may be involved in the pathophysiology of progressive renal disease, which can ultimately lead to cardiovascular disease and increased mortality risk. There are a number of mechanisms through which vitamin D status may affect the progression of renal failure including: control of cell proliferation and differentiation, changes in the loss of podocytes, modulation of inflammation such as the suppression of proinflammatory cytokines, regulation of the renin-angiogenesis system to lower hypertension, decreased risk of anemia, and reduction of PTH levels [98]. Secondary hyperparathyroidism is a well-recognized hallmark of low circulating 25(OH)D even in individuals with normal renal function; however, low serum 25(OH)D in CKD has been shown to aggravate secondary hyperparathyroidism [98].

In the early 1970s, the active form of vitamin D_3, calcitriol, was used in the management of secondary renal hyperparathyroidism in CKD and hemolysis patients. Treatment with calcitriol waned with the publication of the 2009 Kidney Disease Improving Global Outcomes (KDIGO) guidelines, which recommended measurement of 25(OH)D and correction of vitamin D deficiency and insufficiency using 25OHD (calcifediol), cholecalciferol (D_3), or ergocalciferol (D_2) [99]. These treatment recommendations are the same as those for the general population [96] and studies have shown that the use of the precursors of the active form of vitamin D were safer and associated with survival benefits due to their pleiotropic effects on the cardiovascular system of the CKD patients [99]. The use of the active form of vitamin D sterols was controversial with some studies showing reduction in mortality in renal disease patients and no mortality benefits in other studies, particularly at higher doses [100,101].

Systematic reviews and meta-analyses in both dialysis and nondialysis CKD patients show nutritional vitamin D replacement using cholecalciferol or ergocalciferol increase serum 25(OH)D concentrations and effectively reduce PTH without adverse effects of hypercalcemia or hyperphosphatemia [102,103]. The majority of recent clinical trials of nutritional vitamin D in CKD patients have used cholecalciferol [104,105]; however, ergocalciferol's availability in higher doses through prescription have been shown to be safe and effective in lowering PTH in CKD stages III–V [106,107]. The Divine trial (Dialysis Infections and Vitamin D in New England), a prospective, multicenter, double-blind, randomized, placebo-controlled trial compared a high and standard dosing regimen of ergocalciferol with placebo in hemodialysis patients [106]. They reported safe and effective increases in 25(OH)D concentrations after short-term high doses (50,000 IU weekly or monthly) for 12 weeks without significant change in calcium, phosphate, or PTH concentrations over the study duration.

Calcifediol ($25(OH)D_3$) is the least studied of the three nutritional forms of vitamin D available for correction of vitamin D insufficiency/deficiency, and was recently introduced in a modified release formulation [90]. In a study in nondialyzed CKD patients that compared calcifediol treatment to placebo, the oral calcifediol was effective in increasing 25(OH)D in a dose-dependent manner [108]. It is important to observe the recent recommendations of the Italian Society of Nephrology's 2016 Guidelines specific to treatment with oral nutritional forms of vitamin D used to correct vitamin D deficiency in CKD patients. The society warns that: "Regardless of the preferred oral regime used in predialysis CKD patients, treatment with cholecalciferol, ergocalciferol and calcifediol should be discontinued in the presence of

25(OH)D levels [100 ng/mL] and/or with persistent hypercalcemia (> 10.5 mg/mL) in the absence of active vitamin D therapy" [99].

New evidence suggests that none of the three nutritional forms of vitamin D are as effective as exposure to adequate UVB or natural sunlight in CKD [109,110]. Krause and coworkers reported that patients who continued skin exposure to sun-simulating artificial lamps two or three times weekly before routine hemodialysis sessions maintained normal circulating $25(OH)D_3$ and $1,25(OH)_2D_3$ by stimulating the skin's natural ability to activate the conversion of the precursor to vitamin D_3. These authors recommend the use of intermittent suberythemal UVB exposure with a sun-simulation spectrum to treat and prevent vitamin D deficiency in chronic and end-stage kidney disease patients, a noninvasive treatment that could even be carried out during hemodialysis.

Kidney dysfunction is also associated with severe anemia due to low iron availability for hemoglobin synthesis, and with insulin resistance and glucose intolerance in the metabolic syndrome. CKD is associated with higher serum hepcidin (an iron storage protein), low serum 25(OH)D, and anemia, especially in African-American children [111,112]. Correction of vitamin D deficiency lowers hepcidin levels, allowing for improved iron availability, thus correcting the anemia.

Chonchol and Scragg [97] in their analyses of kidney function and vitamin D status in the NHANES III survey also demonstrated that serum 25(OH)D and levels of kidney function (eGFR) were both inversely associated with the homeostasis model assessment of insulin resistance (HOMA-IR), yet were independent of each other. Conversely, they found that survey participants with serum 25(OH)D levels in excess of 81 nmol/L had lower HOMA-IR. These findings suggest that low serum 25 (OH)D may be a risk factor in cardiovascular disease, since insulin resistance is an integral part of the putative path to cardiovascular disease development.

Strategies to raise serum 25(OH)D may decrease the risk of cardiovascular disease [113,114]. Evidence for a role of adequate vitamin D status in slowing the development of cardiovascular disease was demonstrated in a randomized, placebo-controlled, double-blind vitamin D supplementation trial where 2000 IU of cholecalciferol markedly improved the inflammatory cytokine profiles in patients with congestive heart failure [114]. In a recent review, the plausibility of vitamin D's involvement was highlighted [113]. There are vitamin D metabolizing enzymes in the heart and blood vessels, and in animal models of cardiovascular disease, vitamin D is antiatherosclerotic, antiinflammatory, and has direct cardioprotective actions. In epidemiologic studies in the general population, low serum 25(OH)D is associated with increased risk of cardiovascular disease and mortality. Currently, there are few and inconsistent data from RCTs concerning cardiovascular disease prevention and treatment; consequently, a recommendation cannot be made other than ensuring vitamin D adequacy in those at risk for cardiovascular disease [113,114].

4 Vitamin D and Neurological Conditions

As outlined in Table 43.12 there are many conditions for which vitamin D has been shown or has been implied to be important in their pathogenesis. Of interest are associations with conditions affecting the brain, such as dementia, Parkinson's disease, and autism. These diseases do not necessarily share similar pathways, and are only briefly described as less is known about each one. Evidence for a causal relationship between vitamin D deficiency and adverse cognitive or behavioral effects was first presented in 2008 [115]. There is biological plausibility in linking vitamin D to brain development and function but the effects appear to be "subtle."

Dementia, particularly Alzheimer's disease, has become an increasing health problem, in part due to longer life spans, but other causes may be implicated. In a population-based U.S. study, vitamin D deficiency was associated with increased risk of all-cause dementia and Alzheimer's disease. The risk of all-cause dementia and Alzheimer's disease markedly increased below a threshold of 50 nmol/L [116]. Parkinson's disease incidence has been related to outdoor exposure (UVB), giving rise to the hypothesis that vitamin D status may be a causal factor [117]. And finally, evidence is growing that autism is related to vitamin D. Patrick and Ames has shown that vitamin D (calcitriol) regulates serotonin synthesis and that this mechanism may be responsible for increased risk of autism in children whose mothers were vitamin D deficient [118].

D Treatment of Vitamin D Deficiency

When an individual is depleted of vitamin D, the amount of vitamin D needed for repletion is higher than that needed to maintain status; aggressive therapy is needed. Table 43.3 provides a list of commonly used vitamin D supplements, and new vitamin D-related medications are updated continuously at the Food and Drug Administration website [119]. As indicated in Table 43.11, many forms of rickets require high doses of vitamin D. This vitamin D can be provided daily or in intermittent doses, although there has been concern expressed about excessively high doses given monthly or yearly [120]. Hollis and Wagner [120] argue that vitamin D should be available on a daily basis to ensure stable circulating concentrations. When given in yearly doses, the level of 25(OH)D achieved is never known as monitoring, if it is

done, may not reveal the extent of transient toxicity. In general, it is not necessary to keep daily dosages below the tolerable UL for vitamin D, as this DRI is not intended as a barrier for treatment under a physician's care [121]. In situations where the diagnosis of rickets is determined to be a vitamin D-resistant form (Table 43.11), doses of the active metabolite 1,25-dihydroxyvitamin D or an analog with similar properties, is often administered.

V DIETARY REQUIREMENTS

A IOM Recommended Intake Values for Vitamin D, 2011

New reference values for vitamin D that apply to Canada and the United States were announced by the IOM in November 2010 and published in 2011 [4]. These revised DRIs generated much discussion, some of it in support [122], but many critical of the way in which the evidence for new roles of vitamin D were dismissed in the deliberations [123,124]. DRIs provide the reference values for assessment and planning functions in nutrition [121], that is, the EAR which is the median requirement, the RDA which is the amount that meets the needs of almost all (97.5%) healthy persons in the population, the Adequate Intake (AI) which is set for infants age 0–1 year, and the tolerable UL which is set at an intake level that poses no risk for adverse effects. Since first setting DRI recommendations in 1997, evidence has emerged suggesting a need to revise those initial DRI values [124]. The 2011 DRI committee used evidence-based reports and other data in its deliberations. The challenges that the committee identified in setting the new DRIs included: (1) an acknowledgment that vitamin D can be made through skin synthesis, so setting intake values must be made in the context of minimal sun exposure; (2) recognition that studies designed to provide evidence for dietary effects on bone gave both calcium and vitamin D, so isolating vitamin D's effects were a challenge; (3) complications in interpreting the impact of poor vitamin D status as bone health can be maintained with high intakes of calcium making the link between vitamin D and bone health very dependent on usual calcium intakes, which are generally low; and (4) the inability to distinguish the contribution to serum total 25(OH)D of sun-induced synthesis of vitamin D from ingested vitamin D, except in studies where 25(OH)D is measured in winter in subjects who are not exposed to other confounding factors such as trips to sunny destinations or use of tanning beds [4].

The values for each age/sex group for EAR, RDA, and AI are in Table 43.14. The level of 25(OH)D that was defined for adequacy for almost everyone including individuals with darkly pigmented skin (i.e., the RDA) was set at serum 25(OH)D at or above 50 nmol/L. This circulating level of 25(OH)D was deemed to provide for maximal calcium absorption, minimal risk of rickets in

TABLE 43.14 Dietary Recommendations for Vitamin D Intake Based on Healthy and At-Risk Status

Organization and Criterion	Age Group	Recommendation	Level of 25(OH)D Achieved	Notes
Healthy Population				
IOM, 2011	0–1 year	400 IU	50 nmol/L	RDA for healthy Canadians and Americans to achieve a 25(OH)D level of 50 nmol/L of 50 nmol/L. Infant level is an AI
	1–18 years	600 IU		
	19–70 years	600 IU		
	71+ years	800 IU		
At-Risk Population				
Endocrine Society, 2011	0–1 year	400–1000 IU	75 nmol/L	Recommended intake for at-risk patients in the United States to achieve a 25(OH)D level of 75 nmol/L. At-risk includes those who have a vitamin D deficiency, kidney failure, malabsorption syndrome, hyperparathyroidism, on medications that interfere with vitamin D, are African-American or Hispanic, are pregnant or lactating, are older adults with a history of falls or nontraumatic fractures, are obese, or have granuloma-forming disorders
	1–18 years	600–1000 IU		
	18+ years	1500–2000 IU		

Sources: From Institute of Medicine, Dietary Reference Intakes for Calcium and Vitamin D, National Academy Press, Washington, DC, 2011; M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M.H. Murad, C.M. Weaver, Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline, J. Clin. Endocrinol. Metab. 96 (2011) 1911–1930.

children, reduced fracture risk in adults, and minimal risk of osteomalacia in adults [4]. Using data from studies conducted in winter time or at very high latitudes where sun exposure is assumed to be minimal, they used simulated dose–response prediction equations to find the intake of vitamin D that would achieve a mean 25(OH)D level of 50 nmol/L in almost everyone. This intake was found to be 600 IU, irrespective of age, gender, or race/ethnicity. There was recognition that older adults demonstrated a high degree of variability in attaining 25(OH)D in studies, thus their RDA was set at 800 IU. The level to achieve 40 nmol/L was estimated as 400 IU; this is the EAR. The vitamin D recommendation of 400 IU for infants, as an AI, is based on maintaining adequate serum 25(OH)D above 50 nmol/L. Recommended values during pregnancy and lactation are not different from other women in the same age group [4].

B Recommendations for At-Risk Groups

1 Endocrine Society, 2011

The IOM recommendations are for healthy individuals who are free of most chronic conditions and the target level of 25(OH)D used to determine the RDA was 50 nmol/L [4]. Subsequent to the IOM publication of the DRI for vitamin D and calcium, the Endocrine Society also published intake guidelines, but for individuals with chronic conditions known to influence vitamin D status [51]. The Endocrine Committee of experts defined vitamin D adequacy for those at risk as 75 nmol/L 25(OH)D [51]. The Endocrine Society focused on at-risk groups and stated that evidence is available to define "deficiency" as a level of 25(OH)D $<$50 nmol/L. Those individuals with a designation of "at-risk" include: rickets, osteomalacia, osteoporosis, CKD, malabsorption syndromes, medication use that interferes with vitamin D (e.g., see Table 43.13), and granuloma-forming disorders. In addition, there are life stage groups—pregnancy, lactation, older adults with fracture or fall history—and racial/ethnic groups—African-American and Hispanic children and adults—who are included in the "at-risk" designation. Finally, obesity puts people more at risk for vitamin D deficiency. The dietary recommendations for at-risk groups published by the Endocrine Society are found in Table 43.14. Intakes are provided as ranges as it is not known how much cholecalciferol or ergocalciferol is needed to raise serum 25(OH)D levels over 75 nmol/L.

2 Recommendations for Pregnancy and Lactation

By the third trimester of pregnancy there is an elevation of 1,25-dihydroxyvitamin D by the mother, corresponding to the time the fetus begins to calcify bones and the need for calcium is greatest [51]. Overall, pregnant women are at high risk for vitamin D deficiency, and complications such as preeclampsia and cesarean section are increased in those who are deficient [51]. In the 2011 IOM report, no special considerations were made for pregnancy; that is, women during these times would follow the recommendations for nonpregnant or nonlactating women. The IOM justification was that there were no data to suggest the needs of pregnant women were different, for example, fetal outcomes were compromised only when maternal 25(OH)D was below 40 nmol/L, a level that should be met if the woman followed RDA recommendations. The IOM indicated that during lactation, small increases in maternal 25(OH)D do not appear to impact breast milk content or infant 25(OH)D status [4]. However, the 2011 Endocrine Society Guidelines on vitamin D, pregnancy, and lactation are classified as times when screening for 25(OH)D should occur [51]. Recommendations during pregnancy by this group are the same as for at-risk adults of the corresponding age (Table 43.14).

There is emerging evidence that vitamin D recommendations should be made for pregnancy. Evidence is strong for vitamin D insufficiency being associated with first trimester pregnancy loss [125], gestational diabetes [126], preeclampsia [127], preterm birth [128], higher rates of primary Cesarean section [129], small for gestational age infants [130], and maternal postpartum depression [131]. With respect to lactation, it has been shown that when intakes of vitamin D by lactating mothers are optimal, that is, the mother's 25(OH)D levels are $>$75 nmol/L, breast milk levels reach "physiological" levels, supplying 400 IU of vitamin D per day to the infant [70,71]. The amount needed by lactating mothers to give the equivalency of 400 IU per day to the infant is not known but has been estimated to range between 2000 IU [70] and 6400 IU [71].

VI SAFETY OF VITAMIN D

A The Tolerable UL for Vitamin D

The daily tolerable UL was established to discourage potentially dangerous self-medication [121]. The two main indicators of excess vitamin D include hypercalcemia, which can lead to calcification of soft tissues such as arteries (arteriosclerosis) and kidney (nephrocalcinosis), and hypercalciuria, which reflects the presence of excess serum calcium and could be damaging on its own. Kidney stones are sometimes raised as a concern when vitamin D intakes are increased. When incidence of kidney stones is reported from RCTs of vitamin D, there is usually calcium supplementation along with vitamin D, so it has been difficult to distinguish the cause [85]. A study of over 2000 participants who had blood 25(OH)D measured

TABLE 43.15 Comparison of Safety Assessments for Vitamin D Based on Healthy and At-Risk Status

Organization and Criterion	Tolerable UL	
Healthy Population		
IOM, 2011	0–0.5 year	1000
	0.5–1 year	1500
	1–3 years	2500
	4–8 years	3000
	9–13 years	4000
	14+ years	4000
At-Risk Population		
Endocrine Society, 2011	0–0.5 year	2000
	0.5–1 year	2000
	1–3 years	4000
	4–8 years	4000
	9–13 years	4000
	14+ years	10,000

Sources: From Institute of Medicine, Dietary Reference Intakes for Calcium and Vitamin D, National Academy Press, Washington, DC, 2011; M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M.H. Murad, C.M. Weaver, Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline, J. Clin. Endocrinol. Metab. 96 (2011) 1911–1930.

and reported, prospectively, on health outcomes. No statistically significant association between serum 25(OH)D and kidney stones was found. Serum 25(OH)D ranged between 50 and 250 nmol/L [132].

A risk assessment for vitamin D showed that based on an analysis of all published reports related to excess vitamin D ingestion up to 2007, doses of supplemental vitamin D_3 ingested at levels below 10,000 IU/day were not associated with toxicity [133]. It is possible that intakes consistently above 50,000 IU/day could be linked to side effects including hypercalcemia [4].

UL values for infants 0–6 months and 6–12 months were set at 1000 and 1500 IU, respectively (Table 43.15). A no-observable-adverse-effect-level of 1800 IU was used to set these ULs as studies exist where high doses of vitamin D have produced hypercalcemia. An uncertainty factor (UF) of 1.8 was set for very young infants and a smaller UF for older infants indicting greater tolerance to vitamin D. For adults over 18 years, the committee concluded that 25(OH)D should not be above 125–150 nmol/L, and that using published data from a dosing study, determined that 4000 IU would not raise serum 25(OH)D above this threshold [4]. In that dosing study, Heaney et al. [16] gave cholecalciferol to subjects in doses up to 10,000 IU, and found no adverse effects in men treated for 5 months. The IOM indicated that attaining a 25(OH)D above 125 nmol/L had no potential benefit and possible (unknown) risk; therefore, the UL was set at 4000 IU which is an adjustment down from the 5000 IU that subjects ingested to reach 125 nmol/L with 5 months of dosing. For children, the committee chose to "scale down" the adult UL [4].

The Endocrine Society's recommendations for UL values are higher than those of the IOM levels. The Endocrine Society uses these higher amounts, shown in Table 43.14, as they represent intakes that would be used in treatment regimens for those at high risk of vitamin D deficiency or insufficiency [51]. Unless a person is under a physician's care, it would be prudent that the IOM values for ULs should be used as the highest safest intakes.

B Vitamin D Intoxication

Accidental poisoning or uninformed supplementation with vitamin D can occur, causing vitamin D intoxication [4,133]. Intakes reported in nine cases involved levels in the range of approximately 30,000–2,600,000 IU, and durations were between days and decades [133]. The signs and symptoms of vitamin D intoxication are associated with hypercalcemia and include constipation, lethargy, confusion, polyuria, and polydipsia [134]. Generally, toxicity occurred in cases where intake was over 50,000 IU per day, well above the current UL [4] or that set by the Endocrine Society [51]. Serum 25(OH)D associated with these high intakes ranged between 700 and 1600 nmol/L, well above the value of 250 nmol/L considered "high" [133]. The mechanism for toxicity with vitamin D is believed to involve increases in the concentrations of many vitamin D metabolites, including vitamin D itself as well as 25[OH]D; at high concentrations the DBP binding capacity is exceeded and there is release of "free" 1,25[OH]D which enters target cells to act unabated.

There is a genetic disorder called idiopathic infantile hypercalcemia in which symptoms of vitamin D toxicity occur at intakes much lower than those described above. Symptoms include severe hypercalcemia, failure to thrive, vomiting, dehydration, and nephrocalcinosis [135]. The cause of this is now understood to be mutations in CYP24A1, the enzyme responsible for inactivating vitamin D as shown in Fig. 43.4. Even at intakes as low as 500 IU, four patients developed hypercalcemia indicating that there are reasons to be cautious when first initiating treatment of infants with vitamin D. Hypercalciuria also occurs in these patients suggesting monitoring urine excretion should be one way to detect this genetic disorder.

TABLE 43.16 Factors Influencing Outcomes of RCTs Examining Vitamin D's Role in Chronic Disease

Reason	Comment
1. Inappropriate subject selection criteria	Subjects selected without regard to vitamin D status (deficient or insufficient)
2. Statistically underpowered	Small biological differences require large sample sizes; otherwise at risk of type 2 error
3. Uncontrolled additional exposure to vitamin D	Running a trial in a country with fortification with vitamin D (e.g., United States); running studies in summertime; allowing the use of other supplements (e.g., multivitamin)
4. Ineffective doses	Many early trials used 400 IU vitamin D or less
5. Poor treatment compliance	Subject compliance can be low in trials, and/or may not be tracked well
6. Improper dosing regimen (yearly or monthly)	High doses given intermittently do not act the same as a constant daily dosing; trials use these to streamline compliance to treatment
7. Insufficient treatment duration	Serum response to vitamin D treatment requires a minimum of 3 months exposure and likely longer for chronic disease endpoints

Sources: From R. Jorde, G. Grimnes, Vitamin D: the need for more randomized controlled trials, J. Steroid Biochem. Mol. Biol. 148 (2015) 269–274; W. Grant, Re: "Is high dose vitamin D harmful?" Letter to the editor, Calci. Tiss. Res. 92 (2013) 489–490; B.W. Hollis, C.L. Wagner, The role of the parent compound vitamin D with respect to metabolism and function: why clinical dose intervals can affect clinical outcomes, J. Clin. Endocrinol. Metab. 98 (2013) 4619–4628.

VII FUTURE CONSIDERATIONS

As indicated in this chapter, there are many diseases found to be associated with vitamin D status, yet concrete evidence is lacking for many of them. As a result, there are differing opinions about what target serum concentration of total 25(OH)D to use as a cutoff. One reason for such confusion over vitamin D requirements is due to the lack of understanding of how to gather evidence confirming the role of vitamin D in chronic disease risk. Most studies have been cross-sectional, thus supplying only "associations" rather than cause and effect. Some large prospective studies have been done, yet there are many confounders. It is clear that the major problems lie with the design of the RCTs, the so-called gold standard for evidence-based dietary recommendations. Many vitamin D experts are calling for better designed RCTs, citing the numerous ways in which past RCTs have erred [136,137]. Table 43.16 outlines many of the ways in which RCTs involving vitamin D have led to misinterpretation of results. When insufficient doses of vitamin D are given to groups of subjects who are already adequate, it is not surprising there would be null findings. Even the large-scale trials that have not yet reported findings by the time this chapter was written may have design flaws that will contribute to the confusion, making interpretation of requirements for vitamin D inconclusive.

VIII CONCLUSION

Vitamin D inadequacy is a significant public health concern in the United States, as well as other countries around the world, whether it is defined by the IOM as 25 (OH)D $<$50 nmol/L, or by the Endocrine Society at 25 (OH)D $<$75 nmol/L. Many Americans are in high-risk groups, or get little sun exposure, so having an affordable source of food that is relatively low in vitamin D exacerbates this problem. Action is needed to ensure adequate dietary intakes from food and dietary supplements and/or appropriate sun exposure. Vitamin D deficiency leads to not only rickets and osteomalacia, which are likely to go undiagnosed in the population, and to osteoporosis, but also to neuromuscular disabilities, neurological conditions, kidney failure, increased risk of cancer development, poor immunity, and to other problems still under investigation. Better understanding of vitamin D metabolism and status will lead to its effective use in prevention and treatment of a variety of chronic diseases.

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Chapter 44

Osteoporosis: The Early Years

Connie M. Weaver and Kathleen M. Hill Gallant
Purdue University, West Lafayette, IN, United States

I INTRODUCTION

The risk of developing osteoporosis is largely determined by the mass and size of bone acquired by adulthood known as peak bone mass [1]. The greater the skeletal mass at its peak and the stronger the geometry, the greater the amount of loss that can occur before entering the fracture risk zone. An interplay between heritable factors and environmental factors determines peak bone mass (Fig. 44.1). It is estimated that genetics contributes 60−80% and lifestyle factors contribute the remaining 20−40% [2,3]. Genes that control growth and development are thought to also largely determine bone acquisition [2].

Nutrition and physical activity are the primary lifestyle determinants of bone acquisition, and increasing evidence suggests that the interactions between these two are stronger determinants than either alone. Nutrition plays an important role in preventing osteoporosis through building optimal peak bone mass within one's genetic potential. This chapter reviews the influence of diet and nutrients on bone health and the basis of requirements for nutrients that are based on skeletal health. Nutrition likely can also play a role in fracture risk during childhood. Skeletal fragility in childhood occurs when bone mineral density (BMD), as assessed by dual-energy X-ray absorptiometry (DXA), expressed as a *Z*-score is less than −2.0 [4]. Skeletal fragility can arise from a lag between peak height velocity and bone mineralization during the pubertal growth spurt or as a consequence of eating disorders or disease. Less is known about the role of diet in the context of pediatric disorders, and research is needed to address these gaps.

This chapter focuses on the development of peak bone mass and the role of nutrition in bone acquisition. The reader should consult the companion chapter by Robert Marcus (Chapter 45: Osteoporosis in Adults) for a discussion of osteoporosis and bone qualities.

II ACQUIRING PEAK BONE MASS AND BONE STRENGTH

Understanding the timing for an effective nutritional intervention can be as important as the nature of the intervention. Textbooks commonly cite that bone mass is acquired throughout the third decade of life and frequently quote a longitudinal study that averaged annual bone gains over a decade, ignoring the fact that the rate of accrual decreased over time and became trivial by age 30 years [5]. In a metabolic balance study, women older than age 21 years were not in positive calcium retention on intakes of 1300 mg calcium per day [6]. Increase in BMD with age depends on the skeletal site. For total body BMD, 95% of adult peak bone mass was achieved by age 16.2 years and 99% was achieved by age 22.1 years [7]. Peak BMD in white women occurred at age 23 years for the spine, 18.5 years for the femoral neck, 14.2 years for the greater trochanter, and 15.8 years for Ward's triangle [8] (Fig. 44.2). Clearly, to markedly influence peak bone mass, lifestyle practices are more important prior to the end of puberty than postadolescence.

When there are transient periods during which diet is inadequate, some catch-up growth is possible [9]. In a calcium-supplemented controlled trial from early puberty until development of peak bone mass in girls habitually consuming on average 830 mg calcium per day, calcium supplementation increased accrual of BMD of total body (Fig. 44.3) and radius through puberty compared to the placebo group [10]. However, by age 18 years, the advantage was reduced. There was an interaction with size such that girls who were taller by age 18 years did not fully catch up. The extent of catch-up growth undoubtedly

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depends on the degree of inadequacy of the diet, the timing and duration of the period of inadequacy, and the degree of repletion. Regardless, the period of inadequacy is itself a period of vulnerability—that is, in this example, a higher risk of fracture.

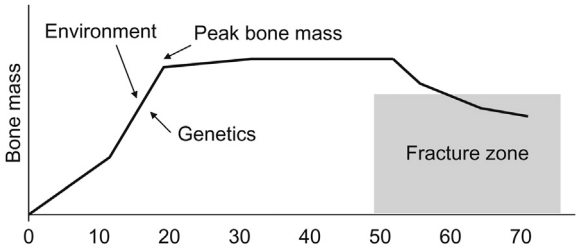

FIGURE 44.1 Bone mass throughout the life span. The influence of genetics and the environment is greatest during growth.

The impact of genetics on development of peak bone mass is evident in Fig. 44.3. The light gray lines show tracking of total body BMD of 15 representative individuals. Those who began the trial with lower BMD remained lower throughout development of peak bone mass. Modifications through lifestyle choices occur within one's genetic potential.

Bone geometry plays an additional role beyond bone mass in resistance to fracture. Bone geometry includes dimensions of bone such as length and cross-sectional area. The relationship of bone geometry to fracture risk in childhood and measurement by peripheral quantitative computed tomography has been described by Kalkwarf [11]. The effect of diet on bone geometry is a rather recent field of study because of the relative newness of this technique. The exercise intervention trials that have used this outcome measure have illustrated additional information gained about bone strength.

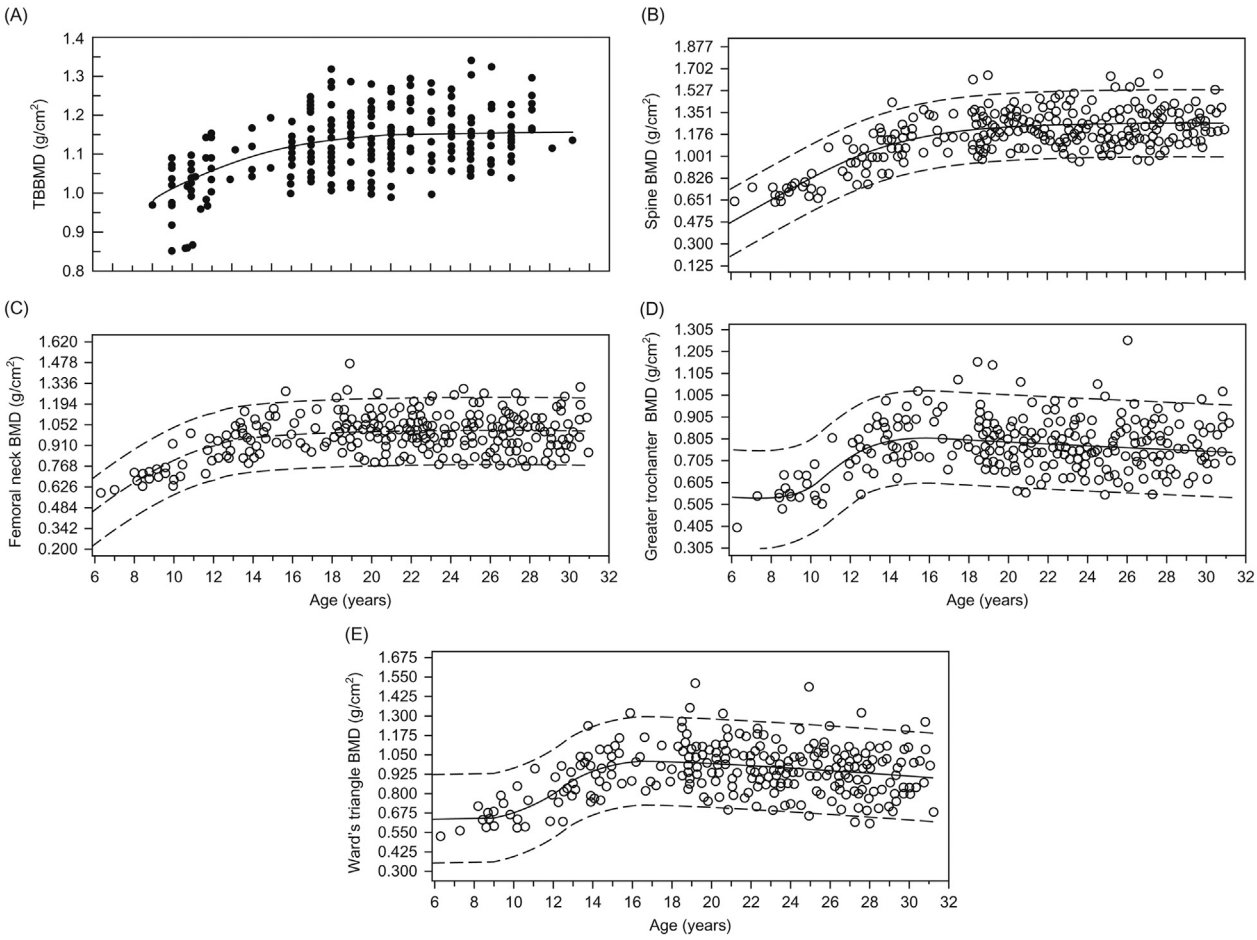

FIGURE 44.2 BMD accumulation with age in females. Gains in BMD vary with skeletal site. Almost 95% of adult peak total body BMD occurred by age 15.2 years (A), and the highest BMD of the spine occurs by age 23 years (B), by age 18.5 years for the femoral neck (C), by age 14.2 years for the greater trochanter (D), and by age 15.8 years for Ward's triangle (E). *Reproduced from D. Teegarden, W.R. Proulx, B.R. Martin, J. Zhao, G.P. McCabe, R.M. Lyle, Peak bone mass in young women, J. Bone Miner. Res. 10 (5) (1995), 711–715 [7]; Y.-C. Lin, R.M. Lyle, C.M. Weaver, L.D. McCabe, G.P. McCabe, C.C. Johnston, Peak spine and femoral neck bone mass in young women, Bone 32 (5) (2003), 546–553 [8].*

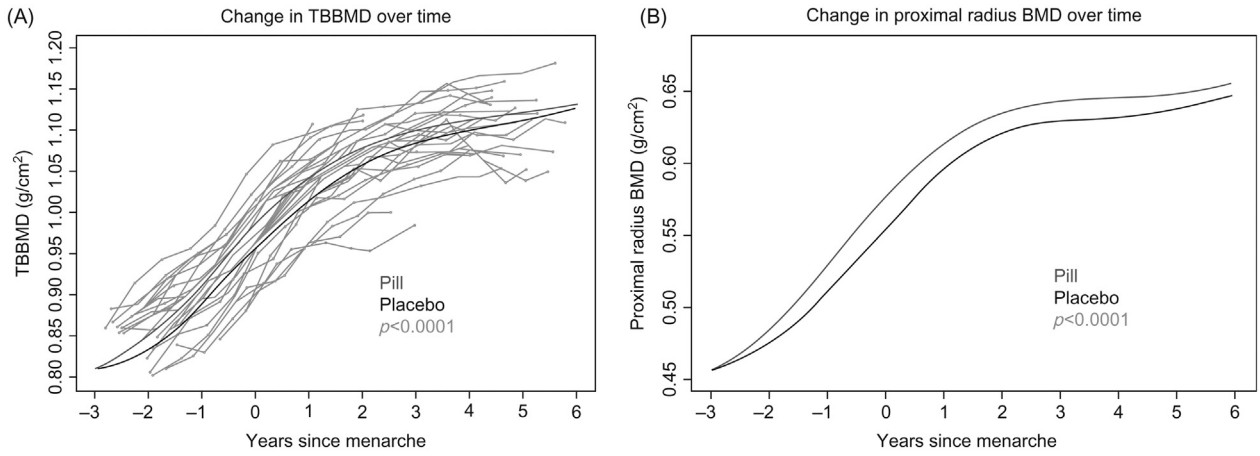

FIGURE 44.3 Total body BMD (A) and proximal radius (B) was significantly higher in prepubertal girls randomized to 1 g calcium supplement daily compared to those assigned to placebo from age 10 to 18 years [10].

III SKELETAL FRAGILITY IN CHILDREN

Fractures in children are associated with low bone mass and density for age [12]. If low bone mass in childhood leads to lower peak bone mass in adulthood, the risk of fracture increases later in life. Relative skeletal fragility can occur naturally with growth spurts. Beyond genetically programmed qualities, bone mass and density throughout life are influenced primarily by nutrition, physical activity, and hormones. Furthermore, eating disorders, smoking, alcohol abuse, and various drugs also influence bone mass. To a large extent, these factors work independently in that one cannot compensate for inadequacy of another. However, dietary calcium and physical activity have important interactions. Finally, some disorders are associated with low BMD.

A Relatively Low BMD During Puberty

Puberty is a period of rapid skeletal growth that is genetically programmed and hormonally driven. The rate of total body bone mass accrual throughout adolescence was determined by Bailey et al. in a longitudinal study of white boys and girls [13]. From this study, we know that approximately 25% of adult peak bone mass is acquired over approximately 2 years—on average this occurs from age 12 to 14 years in girls and age 13 to 15 years in boys. Peak bone mineral content (BMC) velocity is higher and occurs later for boys than for girls. The timing of bone mineral acquisition is more closely linked to pubertal development than to chronological age [2,13].

During puberty, bones first elongate and then mineralization, or bone consolidation, ensues. At the age of peak height velocity, adolescents have acquired 90% of their adult height (or bone size) but only 60% of adult total body BMC. Thus, early puberty is a period of relatively low BMD and, therefore, susceptibility to fracture is not unlike that of age-related bone loss [14–16]. The higher incidence of fracture during this time of life corresponding to the lower BMD in the study by Bailey et al. [13] is shown in Fig. 44.4. Approximately 51% of boys and 40% of girls experience fractures by age 18 years [16].

The dramatic increase in rates of childhood fracture in the United States in just three decades is apparent in Fig. 44.5. Fracture incidence increased 32% in males, with the greatest increase at age 11–14 years, and 56% in girls, with the greatest increase at age 8–11 years. Increased rates of childhood fracture may relate to reduced consumption of milk, change in physical activity or recreational activities, and/or increased body weight during this time period. The prevalence of excessive adiposity in children and adolescents has nearly tripled while the incidence of fracture has increased [19]. In adults, increased weight has been associated with increased bone mass [20], but overweight children and adolescents have higher rates of fracture [12,21,22]. The increased incidence in fracture with excessive body weight in children has been hypothesized to occur because of greater force being placed on bones such as the radius during falls, lower bone mass and bone strength with increasing body fat when adjusted for total body weight, and impaired mobility [21,23–28]. The interaction between calcium intake and body mass index (BMI) is described later. Changes in bone geometry that accompany increases in bone size throughout childhood include increases in cortical thickness and bone diameter [29]. Bone diameter and cortical thickness are less in children with excess body fat [28]. This emphasizes the need to maintain ideal body weight in children.

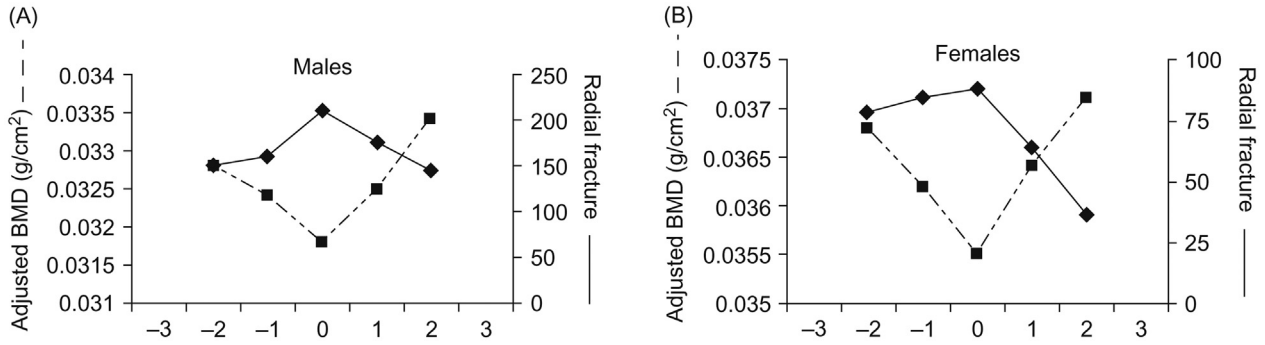

FIGURE 44.4 Distal radius fracture incidence for (A) boys and (B) girls from local hospital admissions compared with the total body BMD adjusted for body size aligned by biological age (years from peak height velocity) from the Bailey et al. study [13]. *Adapted from R.A. Faulkner, K.S. Davison, D.A. Bailey, R.L. Mirwald, A.D.G. Baxter-Jones, Size-corrected BMD decreases during peak linear growth: implications for fracture incidence during adolescence, J. Bone Miner. Res. 21 (2006) 1864–1870 [17] with permission.*

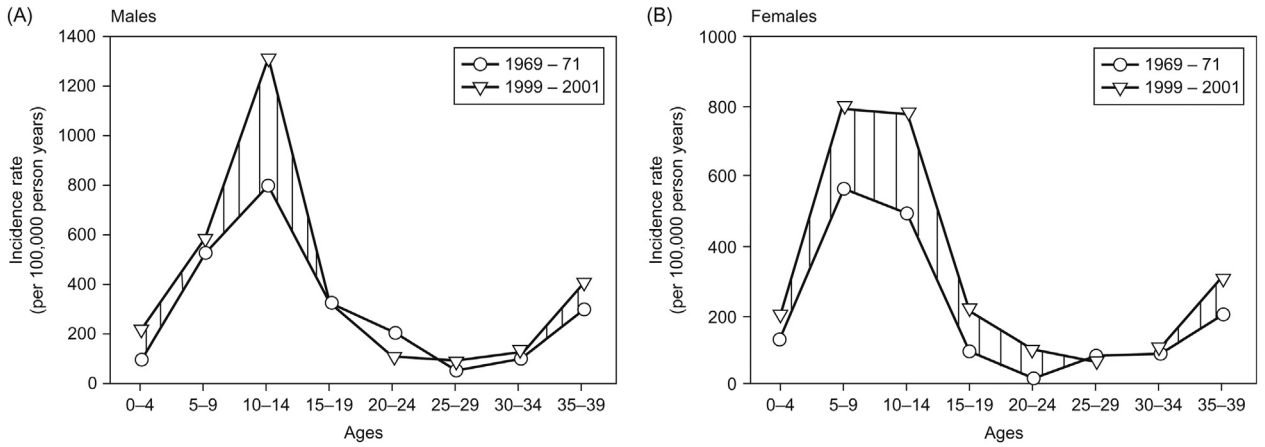

FIGURE 44.5 Childhood forearm fracture incidence in males (A) and females (B) from 1969 to 1971 (lower line) and 1999 to 2001 (upper line) in Rochester, Minnesota. *Reproduced from R.P. Heaney, C.M. Weaver, Newer perspectives on calcium and bone quality, J. Am. Coll. Nutr. 24 (6) (2005) 574S–581S [18] using data from S. Khosla, I.J. Melton, M.B. Delatoski, Incidence of childhood distal forearm fractures over 30 years, JAMA 290 (2003) 1479–1485 [14].*

B Disorders Associated With Low Bone Mass

A number of disorders have been associated with low bone mass in children (Table 44.1). Skeletal health should be evaluated with diagnosis of these disorders. If skeletal fragility develops, bone fractures can occur with minimal or no trauma, a condition known as osteoporosis. The ability to reverse bone deficits with early diagnosis and treatment remains uncertain.

Amenorrhea most often results from excessive exercise or energy intakes too low to sustain physiologic levels of estrogen. Anorexia nervosa is an eating disorder characterized by intense fear of gaining weight or becoming fat. In this condition, body weight is less than 86% of normal for height and age. Reduction in bone gains or acceleration of bone loss resulting in low BMD for age can occur with anorexia nervosa [30]. Osteoporosis that develops as a result of anorexia nervosa is more severe than with other causes of estrogen deficiency. Girls with anorexia nervosa had lower fat and energy intakes than did girls without anorexia nervosa [30]. Severity of low bone mass (i.e., osteopenia) is worse if the eating disorder is initiated in adolescence than in adulthood and worse for a longer duration [32].

Little is known about specific diet therapies and improved bone health in the other disorders. Nutrient recommendations or dietary guidelines have not been established for any of these disorders for lowering risk of low bone mass. It is logical that recovery of low body weight would be helpful to bone. Some interesting associations suggest that future research may show a benefit for nutritional therapies. For example, vitamin K deficiency is prevalent among children with cystic fibrosis [31].

IV NUTRITION AND DEVELOPMENT OF PEAK BONE MASS

The role of diet in development of peak bone mass is thought to have a great impact on risk of osteoporosis [1].

TABLE 44.1 Pediatric Conditions Associated with Low Bone Mass

Amenorrhea
Anorexia nervosa
Brain tumors (craniospinal irradiation)
Celiac disease
Congenital hypothyroidism
Cystic fibrosis
Diabetes mellitus
Epilepsy
Glucocorticoid-sensitive nephrotic syndrome
Hemophilia
HIV/antiretroviral therapy
Inflammatory bowel disease
Liver transplantation
Osteogenesis imperfecta
Renal disease and transplantation
Rheumatologic disease
Turner's syndrome

Source: Derived from C.M. Gordon, Evaluation of bone density in children, Curr. Opin. Endocrinol. Diabetes 12 (2005) 444–451 [29].

The formative years set the foundation for the skeletal reserves and for lifelong eating and exercise habits. In fact, osteoporosis has been called a pediatric disease. This chapter approaches the role of diet under two themes: diet patterns and individual nutrients. So much of the research base has focused on individual nutrients that supplementation with calcium and vitamin D has become a first-line strategy of prevention and therapy. Although calcium and vitamin D are the two nutrients important to bone most likely to be deficient, this reductionist approach to research and medical nutrition therapy aimed at building strong bones is woefully inadequate. Dietitians can play a critical role in assessing the overall diet and making recommendations that will have greater impact on body weight and health than simply recommending supplements. To understand the quality of the evidence behind public health recommendations related to bone health, a discussion of the limitations of our research designs and measures is warranted.

A Limitation in Methodology

Whether interpreting the effects of diet patterns or individual nutrients on bone, the limitations of investigation of nutritional effects on bone should be appreciated. Difficulty in assessing diet and individual nutrient consumption is not unique to studies of bone and is discussed elsewhere in this book. To quantitatively determine the effect of intake of a nutrient or diet pattern is best accomplished through controlled feeding studies. However, to control diets for sufficiently long periods for bone properties to change is not practical except in animal studies. Although changes in bone properties occur faster in pediatric studies than in studies of adults, it still requires a year or several years to evaluate the magnitude of the effect of interventions on bone. During the first 6 or more months following onset of an intervention, changes in bone reflect the bone remodeling transient [32]. This is a period of adaptation through changes in bone formation rate or bone resorption rate if the intervention is effective. Longer periods are required to determine the effect of the intervention on steady-state bone balance. In children, changes in bone are relatively rapid during periods of active growth. Often, the major challenge is to distinguish between the effects of intervention and growth [33].

The traditional outcome measures for assessing nutritional effects on bone properties—that is, BMC and BMD—may be altered by 2% or less per year as a result of a nutritional intervention. This magnitude of change is difficult to detect by DXA in short-term studies. However, this level of impact over many years can have a profound effect on risk of fracture. Other changes in bone that can indicate strength, such as bone geometry that can be assessed by three-dimensional imaging, also require substantial intervention periods and, to date, do not offer improved precision over DXA. It is preferable to use BMC rather than BMD as the primary outcome measure to assess efficacy of interventions in children. Effective dietary interventions could augment bone size rather than merely affect the mass of bone per unit volume. Thus, because BMD is calculated to adjust for size, it can miss most of the actual change in bone mass during growth. However, DXA only captures two-dimensional space, and consequently it is somewhat affected by size [34] and can correlate with changes in BMC. Interventions during growth that influence bone mass and bone size have a dramatic influence on bone strength [11]. A number of investigators have attempted to correct DXA measures for bone size, such as calculating bone mineral apparent density, BMC for height, and BMC for bone area, but there is no consensus regarding the best approach [35, 36]. Currently, it is recommended to use BMC as the primary outcome, and it is also helpful to measure bone area and other indicators of bone size. Anthropometric measures can be good predictors of BMC in children [37].

Several shorter term approaches for assessing perturbations in bone are available. Serum and urine biochemical markers of bone turnover can determine qualitative changes in bone turnover, but within-subject variation is high [38]. Furthermore, biochemical markers of bone turnover do not measure bone mineral but, rather,

measure fragments of collagen in connective tissue that leak into circulation when bone remodels. Thus, the units cannot be converted into units of bone.

Other short-term methods for analyzing perturbations in bone most often measure calcium as a surrogate for bone. Calcium is a good surrogate for bone because it is present in a fixed proportion of bone mineral. By multiplying BMC determined from DXA by the fraction 0.31, one can derive calcium content in grams. Calcium levels in the blood are not a good indicator of calcium/bone status because they are tightly controlled within a narrow range. Short-term changes in bone balance can be estimated from calcium balance studies. During growth, bone balance is positive. The use of calcium balance studies to determine requirements and response to an intervention has been described [39]. In children, adaptation to a new calcium intake requires approximately 1 week before balance is determined. Calcium isotopic tracers can be used to measure all components of calcium metabolism [39–41]. Combining calcium balance studies and use of oral and intravenous tracers of calcium can provide the data for compartmental modeling whereby determination rates of transport of calcium in and out of pools and the mass of each pool are possible.

B Strength of the Evidence for Diet and Peak Bone Mass

A recent National Osteoporosis Foundation (NOF) position paper reported a systematic review of predictors of peak bone mass [1]. The consensus grades of evidence for factors related to diet are given in Table 44.2.

TABLE 44.2 Evidence Grading for Nutrition and Development of Peak Bone Mass

Nutrition Factor	Grade
Macronutrients	
• Fat	D
• Protein	C
Micronutrients	
• Calcium	A
• Vitamin D	B
• Micronutrients other than calcium and vitamin D	D
Food Patterns	
• Dairy	B
• Fiber	C
• Fruits and vegetables	C
• Detriment of cola and caffeinated beverages	C
Use of alcohol	D

Source: Adapted from C.M. Weaver, C.M. Gordon, K.F. Janz, H.J. Kalkwarf, J.M. Lappe, R. Lewis, et al., The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations, Osteoporos. Int. 27 (4) (2016) 1281–1386 [1].

C Dietary Patterns and Bone Health

When formulating recommendations for food patterns for different subgroups, the Dietary Guidelines Advisory Committee for Americans (DGAC) considered bone health [42]. The food patterns were designed to meet nutrient recommendations including bone-related nutrients. Calcium and vitamin D were identified as shortfall nutrients of public health concern. The evidence for a relationship between milk and milk products and bone health was also reviewed in determining quantities to recommend for optimal health. In addition to getting adequate nutrients and milk products, the committee considered dietary habits detrimental to health, especially with regard to energy excess but also for bone health. Dietary patterns as they influence acid–base balance may also play a role in bone health. The 2015 DGAC concluded that there is limited evidence for a dietary pattern that is higher in dairy products, fruits, vegetables, grains, and nuts, and lower in meats and saturated fat are beneficial for bone health outcomes in adults (which included decreased risk of osteoporosis and fracture). However, the DGAC could not draw the same conclusion in children and adolescents due to the paucity of data on dietary patterns and bone health in this age group [42].

1 Milk and Milk Products

The NOF position paper rated evidence for the relation of dairy intake to development of peak bone mass as a B grade [1]. Few randomized controlled trials (RCTs) have been done in children with bone outcome measures. The largest and most effective was in Chinese girls with low calcium intakes compared to Western countries [43].

The *Dietary Guidelines for Americans* includes 2 cups of milk or milk product daily for children aged 2–8 years and 3 cups after age 8 years [42]. The amount of milk was set to help meet requirements for several nutrients, including calcium, magnesium, potassium, riboflavin, and vitamin D. Milk products provide approximately 50–79% of the calcium in the diets for children in the food patterns for age and gender recommended by the 2010 DGAC [44]. The milk group also provides more than 10% of the nutrients in the pattern for riboflavin, vitamin B_{12}, vitamin A, thiamin, phosphorus, magnesium, zinc, potassium, protein, and carbohydrate. If milk products are excluded from the patterns, intake for calcium falls below 64% for all children and below 33% for young children; it also falls below 88% for magnesium—as low as 33% for some age groups [44]. Alternatives to milk products given in

the DGAC report were low-lactose milk products [44]. Although some fortified foods, such as calcium-fortified soy milk, have nutrient profiles similar to that of milk, it is difficult to meet calcium and potassium requirements without milk. Milk product consumption has been associated with overall diet quality. Adequacy of milk intake has been associated with adequacy of calcium, potassium, magnesium, zinc, iron, riboflavin, vitamin A, folate, and vitamin D for children [45].

Many calcium-fortified foods are on the market that could theoretically be used to provide the requirements for this nutrient. Gao et al. [46] evaluated the ability of dairy-free diets to meet calcium intake while meeting other nutrient requirements using diets in U.S. children aged 9–18 years for those participants in NHANES 2001–02 who reported no intake of dairy. Calcium requirements were not met without use of calcium-fortified foods, and only one child accomplished this. Average calcium intake without dairy products was 498 mg/day for girls and 480 mg/day for boys compared to 866 and 1070 mg/day, respectively, with dairy products. At calcium intakes of approximately 400 mg/day, calcium retention was only 131 mg/day compared to almost three times that much if the adequate intake for calcium is met [47]. In a longitudinal study of 151 white girls, dairy product/calcium intake at age 9 years was associated with total body BMD gain from age 9 to 11 years [48]. Milk avoiders have increased risk for prepubertal bone fractures [49]. Retrospective studies have shown that the incidence of postmenopausal fracture is inversely related to drinking milk in childhood [50,51]. In the nationally representative NHANES database, the incidence of hip fracture was twice as high for those who consumed one glass of milk or less per week compared to those who consumed at least one serving per day during childhood [50].

Milk consumption in children has declined over time. In the Bogalusa Heart Study, during the two decades from 1972 to 1994, average milk consumption by 10-year olds declined by an average of 64 g [52]. Fluid milk consumption was negatively correlated with soft drink consumption, which had a detrimental effect on bone gain in girls [53]. The displacement of milk with soft drinks removes a rich package of nutrients from the diet. On average, males of all ages consume more dairy than females. In an analysis of NHANES data from 2001 to 2008, females had the highest average dairy consumption at ages 4–8 years old, of around 2 cups/day, that steadily declined with greater age; whereas males had a peak mean consumption of approximately 2.5 cups/day of dairy at ages 14–18 years, that then steadily declined after that point with increasing age [54].

2 Plants Versus Animal-Based Diets

The mix of animal- and plant-derived foods in the diet of an individual influences two postulated determinants of bone health—acid–base balance and amount of protein. Although dietary protein is a nutrient, because the type of protein influences acid–base balance, dietary protein is discussed in this section on dietary patterns. Intake of fruits and vegetables also influences the acid–base balance.

The role of the type and amount of proteins in bone health has been studied for several decades, but little work has been done in children. Alkaline dietary salts contain the cations K^+, Ca^{2+}, and Mg^{2+}, which act as buffers for organic acids produced during metabolism and hepatic oxidation of S-containing amino acids that would otherwise lower blood pH. Increased metabolic acidosis has been associated with increased bone resorption in cell culture systems [55] and increased urinary calcium excretion in humans [56]. Bone is thought to serve as a reservoir of buffering capacity due to the carbonate and hydroxyapatite salts. Typical acid loads produced on a Western diet are on the order of 1 mEq of acid/kg/day. Investigators have attempted to estimate the renal acid load of diets as a measure of acid–base load by taking into account the mineral and protein composition of foods [57]. Thus, diets high in fruits and vegetables that contain potassium and produce an alkaline ash and those richer in plant proteins than animal proteins, which contribute more S-containing amino acids, have been promoted for better bone health through improving acid–base balance. A high ratio of dietary animal to vegetable protein intake has been associated with increased rates of bone loss and increased risk of fracture [58].

The hypothesis that increasing dietary protein or animal versus plant proteins or even acid–base balance influences bone has been challenged. Bonjour [59] argues that bone is unlikely to be the main source of buffering acid loads because bone mineral is not in direct contact with systemic circulation. Rather, buffering of acid loads is accomplished through elimination of carbon dioxide by the lungs and hydrogen ions by the kidney. The increased urinary calcium with increased protein intake generally, or S-containing amino acids specifically, is offset by, and in fact is due to, increased calcium absorption with no increase in bone resorption or net differences in calcium retention [60,61]. Increased dietary potassium does reduce urinary calcium excretion, but this did not appear to affect calcium balance because calcium absorption was also reduced as dietary potassium increased [62]. Intake of fruits, vegetables, and herbs does inhibit bone resorption, but this effect is independent of their alkali or potassium contributions [63].

There is evidence that adequate dietary protein promotes bone accrual in children. In an 18-month RCT in which 12-year-old girls consumed a pint of milk a day, an increase in total body BMD was associated with an increase in serum insulin-like growth factor-1 (IGF-1) [64]. The relation between protein intake and bone gains in lumbar spine and femoral neck in 193 subjects aged 9–19 years was positive, particularly in prepubertal children [65]. An interesting hypothesis has been put forth that aromatic amino acid intake may induce an increase in calcium absorption and

serum IGF-1 compared with branched-chain amino acids through activation of calcium sensor receptors in the gut to increase gastric acid production [66].

A study that related biomarkers of diet over 4 years to bone strength in children indicated that both anabolic and catabolic actions influenced bone strength [67]. Urinary nitrogen reflecting protein intake was positively associated with BMD and measures of bone size. Conversely, potential renal acid load (PRAL) was negatively associated with BMC and cortical area. Dietary protein contributes to PRAL.

3 Salt

Dietary salt is the largest dietary predictor of urinary calcium excretion [68]. However, the response of adolescents to dietary salt is racially dependent. In controlled feeding studies using a crossover design with high (4 g, 172 mmol) and low (1.3 g, 5.7 mmol) sodium diets, white adolescent girls excreted more sodium on a high-salt diet than did black girls of matched weight and sexual maturity [69]. Because calcium is excreted with sodium through the shared transport proteins in the kidney, high-salt diets resulted in more calcium excretion and lower calcium retention in white girls than in black girls in the same study [70]. Thus, a high intake of dietary salt is detrimental to growing bone, but the consequences to bone are greater for white than black individuals.

D Individual Nutrients and Bone Health

Calcium is by far the most studied single nutrient related to bone health. Vitamin D is the focus of much current research. These two nutrients are the most likely ones of those important to bone health to be deficient. It should be understood that because bone is a living tissue, all essential nutrients are required for bone growth.

1 Calcium

a Current Recommended Requirements and Their Basis

Current recommended dietary allowances (RDAs) and upper levels (ULs) for calcium, vitamin D, phosphorus, and magnesium are given in Table 44.3 [71].

Calcium requirements can be determined by using the factorial approach (replacement of losses through urine, stools, and skin adjusted for absorption) or by estimating the intake for maximal calcium retention. The 2010 panel used the factorial approach [71] and the 1997 panel used the intake for maximal retention approach where possible [72], but recommended intakes for most age groups by both panels were the same.

Determining calcium intakes for maximal calcium retention requires studies on a range of calcium intakes. The RCTs described next studied only two levels of calcium intake—that of the self-selected diet or one's dietary intake plus the calcium intervention source. One can assess whether calcium supplementation is effective with this study design, but it is not possible to determine an optimal intake. It would be desirable to estimate bone accretion over a range of calcium intakes in different age groups, but this would require large, expensive studies. Instead, data on calcium retention at different calcium intakes are available from short-term controlled-feeding studies in which composites of diet and complete urine and stool collections are analyzed for calcium so that

TABLE 44.3 Dietary Reference Intakes for Bone-Related Nutrients in Children and Adolescents

	Nutrient							
	Calcium (mg/day)		Vitamin D (μg/day)[a]		Phosphorus (mg/day)		Magnesium (mg/day)	
Life-stage group	RDA	UL	RDA	UL	RDA	UL	RDA	UL
0–6 months	200[b]	ND	10	25	100[b]	ND	30[b]	ND
7–12 months	260[b]	ND	10	37	275[a]	ND	75	ND
1–3 years	700	2500	15	62.5	460	3000	80	65[b]
4–8 years	1000	2500	15	75	500	3000	130	110[c]
9–13 years	1300	3000	15	100	1250	4000	240	
14–18 years	1300	3000	15	100	1250	4000		
Females							350[c]	350[c]
Males							350[c]	350[c]

[a] *10 μg = 400 IU.*
[b] *Adequate intake.*
[c] *Supplementary, not in food.*
ND, not determined; RDA, recommended dietary allowance; UL, upper level.

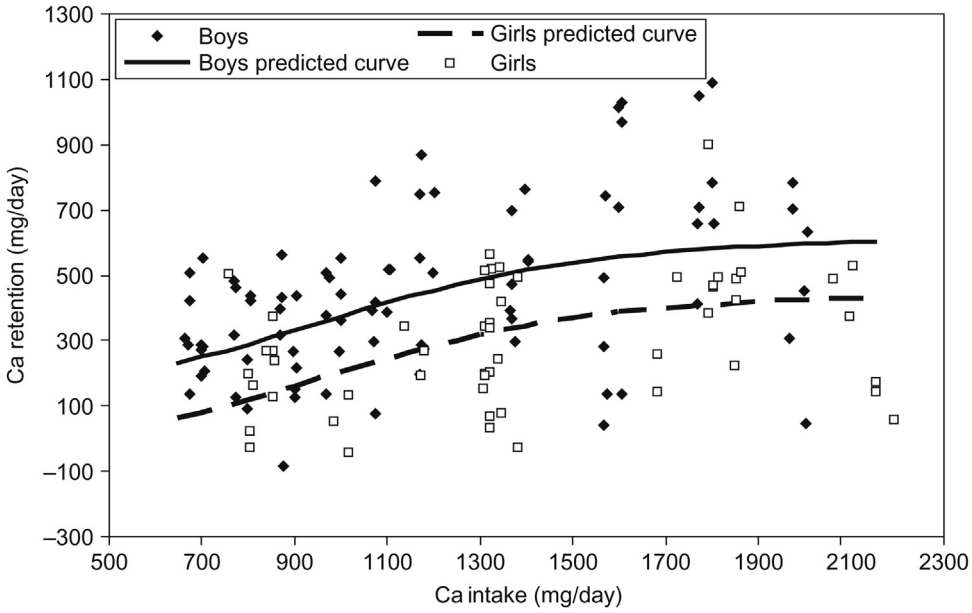

FIGURE 44.6 Calcium retention as a function of calcium intake in adolescent boys (upper curve) and girls (lower curve) [74].

calcium retention or balance (intake—loss from urine, stools, and sometimes sweat) can be calculated. As calcium intakes increase, calcium retention increases until a plateau intake is reached at which further calcium intakes are excreted. A comparison of calcium retention as a function of intake between boys and girls is shown in Fig. 44.6 [73]. Although boys retained more calcium at a given level of calcium intake than did girls, the calcium intake at which retention was not further significantly increased did not differ by gender. Similarly, black girls had higher calcium retention across a range of calcium intakes without different slopes [74]. Thus, boys are more efficient than girls in using calcium, and blacks are more efficient than whites, which results in higher bone mass as adults; however, the need for calcium to produce skeletons of greater bone mass is not detectably different over short periods. Asian girls are also more efficient than white girls in retaining calcium across a range of calcium intakes [75].

b Calcium and BMI

There is an interaction between calcium intake and BMI on calcium retention. Fig. 44.7 illustrates this relationship for white 12-year-old girls [76]. As BMI increases, skeletal calcium accretion increases, but only if calcium intakes are adequate to provide the necessary structural materials. Without adequate dietary calcium, insufficient calcium is retained to build enough skeletal mass appropriate for body weight, which may explain the doubling of fractures shown in Fig. 44.5.

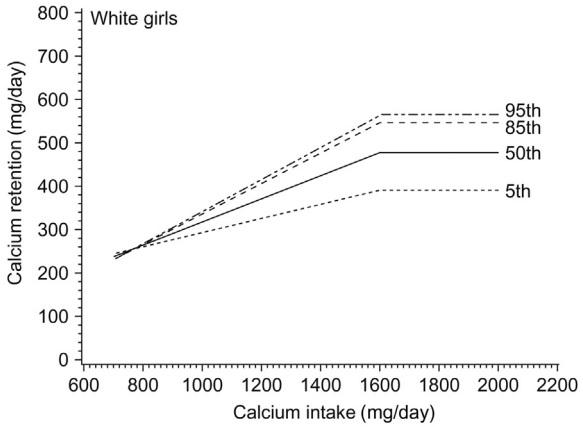

FIGURE 44.7 Influence of BMI (as percentiles of BMI-for-age) on calcium retention curve in white 12-year-old girls [77].

c Evidence for Relationship to Bone

The NOF position paper rated evidence for calcium and development of peak bone mass as grade A [1]. This was the only dietary factor that merited an A grade. This is because there is a large body of evidence from RCTs of calcium intake in children that have reported positive effects on one or more skeletal sites (Table 44.4). Intervention studies have used calcium salts, calcium-fortified foods, and milk with a variety of bone outcomes. Cheese was more effective than calcium carbonate at increasing tibial cortical thickness [89].

Retaining a high-calcium intake during growth for optimizing peak bone mass is prudent. The limitations of

TABLE 44.4 Differences in Mean Changes in BMC and BMD in Calcium Treated Versus Placebo Groups in RCTs in Children

Source	Ref. No.	Subj. No.	Age (Years)	Sex	Race/ Location	Length Study (Months)	Calcium Intake, Controls (mg/day)	Calcium Intake, Treatment (mg/day)	Site	Measure	Group Mean Increase	
											Treatment (T)	Placebo (P)
Johnston et al. (1992)	[77]	140 twins	6–14	F/M	White, IN	36	908	1612	Midshaft radius	BMD	17.7%	15.2%
									Distal radius	BMD	21.5%	18.2%
									Lumbar spine	BMD	20.1%	19.5%
									Femoral neck	BMD	15.3%	14.9%
									Ward's triangle	BMD	15.4%	14.2%
									Greater trochanter	BMD	18.1%	17.11%
Lloyd et al. (1993)	[78]	94	11.9 ± 0.5	F	White, PA	18	960	1314	Total body	BMC	9.6%	8.3%, $p = 0.003$
									Spine	BMC	2.9%, $p = 0.03$	4.7%, $p = 0.05$
Chan et al. (1995)	[79]	48	9–13	F	White, UT	12	728	1437	Total body	BMC	14.2 ± 7.0%	7.6 ± 6.0%, $p < 0.001$
Lee et al. (1994)	[80]	109	7	F/M	Asian, China	18	571	1363	Lumbar spine	BMD	22.8 ± 6.9%	12.9 ± 8.3%, $p < 0.001$
									Distal radius	BMC	15.92 (T) vs 14.95% (P) gain, $p = 0.53$	
										Area	7.74 (T) vs 6.00% (P) gain, $p = 0.081$	
									Lumbar spine	BMC	20.92 (T) vs 16.34% (P) gain, $p = 0.035$	
										Area	11.16 (T) vs 8.71% (P) gain, $p = 0.049$	
									Proximal femoral neck	BMC	24.19 (T) vs 23.42% (P) gain, $p = 0.37$	
Cadogan et al. (1997)	[64]	82	12.2	F	White, Sheffield, UK	18	753	1125	Total body	BMD	9.6 (T) vs 8.5% (P), $p = 0.017$	
										BMC	27.0 (T) vs 24.1% (P), $p = 0.009$	
Bonjour et al. (1997)	[97]	149	7.9 ± 0.1	F	White, Geneva, CH	12	916	1723	Radial metaphysics	BMD	16 ± 3 (T) vs 9 ± 2 g/cm^2 (P), $p < 0.08$	

Nowson et al. (1997)	[81]	87	10–17	F	White, Australia	18	692	>1600	Spine	BMD	1.62 ± 0.84% (T vs P)
									Hip	BMD	NS
									Femoral neck	BMD	22 ± 4 (T) vs 13 ± 4 g/cm^2 (P)
									Femoral diaphysis	BMD	66 ± 3 (T) vs 54 ± 4 g/cm^2 (P), $p < 0.01$
									Lumbar spine	BMD	25 ± 3 (T) vs 23 ± 3 g/cm^2 (P)
Dibba et al. (2000)	[82]	160	8.3–11.9	F/M	Black, Gambia	12	342	1056	Midshaft radius	BMC	3.0 ± 1.4% (T–P), $p = 0.034$
										BMD	4.5 ± 0.9% (T–P), $p < 0.0001$
Moyer-Mileur et al. (2003)	[83]	71	12	F	White, UT	12	865	1524	pQCT of distal tibia	BMC	4.1 (T) vs 1.6% (P), $p < 0.006$
										vBMD	1.0 (T) vs −2.0% (P), $p < 0.006$
Rozen et al. (2003)	[84]	100	14 ± 0.5	F	85 Jewish/ 26 Arab, Haifa, Israel	12	480	1110	Total body	BMC	4.63 (T) vs 4.65% (P), NS
										BMD	3.80 (T) vs 3.07% (P), $p < 0.05$
									Lumbar spine	BMD	4.52 (T) vs 3.95% (P), NS
										BMC	3.66 ± 0.35 vs 3.00 ± 0.43% (P), $p < 0.05$
									Femoral neck	BMC	4.30 (T) vs 3.00% (P), NS
										BMD	2.00 (T) vs 1.39% (P), NS
Stear et al. (2003)	[85]	144	17.3 ± 0.3	F	Cambridge, UK	15.5	938	+1 g Calcium supplement	Whole body	BMC	0.8 ± 0.5% (T vs P), $p < 0.01$
									Lumbar spine	BMC	0.9 ± 0.8% (T vs P), $p < 0.001$
									Hip	BMC	5.3 ± 1.0% (T vs P), $p < 0.001$
Cameron et al. (2004)	[86]	102	10.3 ± 1.5	F	White, Australia	2.4	715	+1200	Total body, lumbar spine, femoral neck, total hip	BMD	NS
Mølgaard et al. (2004)	[87]	113	12–14	F	White, Denmark	12	2 groups, 1717/1320	2 groups, 953/ 660	Whole body	BMC	0.8% (T vs P), $p = 0.049$
											0.5% (T vs P), $p = 0.08$
Prentice et al. (2005)	[88]	143	16–18	M	White, UK	13	1283	1858	Whole body	BMC	1.3% (T vs P), $p = 0.02$
									Lumbar spine	BMC	2.5% (T vs P), $p = 0.004$
									Total lumbar spine	Bone area	1.5% (T vs P), $p = 0.0003$
									Total hip	BMC	23% (T vs P), $p = 0.01$
									Femoral neck	BMC	2.4% (T vs P), $p = 0.01$
									Intertrochanter	BMC	2.7% (T vs P), $p = 0.01$

(*Continued*)

TABLE 44.4 (Continued)

Source	Ref. No.	Subj. No.	Age (Years)	Sex	Race/ Location	Length Study (Months)	Calcium Intake, Controls (mg/day)	Calcium Intake, Treatment (mg/day)	Site	Measure	Group Mean Increase	
											Treatment (T)	Placebo (P)
Matkovic et al. (2005)	[10]	354	10.9 ± 0.9	F	White, OH	48	830	1498	Whole body	BMD	0.215 vs 0.204 g/cm^2, $p < 0.0006$	
									Distal radius	BMD	0.106 vs 0.092 g/cm^2, $p < 0.0026$	
						84			Whole body	BMD	0.268 vs 0.263 g/cm^2, $p < 0.0006$	
									Distal radius	BMD	0.171 vs 0.165 g/cm^2, $p < 0.0006$	
Cheng et al. (2005)	[89]	195	10–12	F	White, Finland	24	1566	671	Whole body	BMC	34.7%	35.5%
									Femoral neck	BMC	24.0%	22.4%
									Total femur	BMC	33.6%	33.6%
									Spine	BMC	46.9%	47.0%
									Tibia cortical thickness	pQCT	31.7%	31.1%
Lambert et al. (2008)	[90]	196	11–12	F	United Kingdom	18	636	1174	Whole body	BMC	25.2%	22.9%, $p < 0.001$
										BMD	0.09%	0.07%, $p < 0.001$
									Total hip	BMC	NS	
										BMD	1.14%	0.16%, $p = 0.002$
									Spine	BMC	0.37%	0.32%, $p < 0.01$
										BMD	0.16%	0.14%, $p < 0.001$
Green and Naughton (2011)	[91]	40	9–13	F	Australia	6	763–786	800		4% location Trabecular vBMD (mg/mm^3) Trabecular area (mm^2) Subcortical density (mg/mm^3) Subcortical area (mm^2) SSI (mm^3)	Radial difference in percent gain between groups 5.2 ± 1.96, $p < 0.05$ 5.4 ± 1.33, $p < 0.05$ 3.4 ± 0.82 0.8 ± 0.07 6.6 ± 1.26, $p < 0.05$	
Khadilkar et al. (2008)	[92]	214	8–12	F	India	12	–	1000	Total body	BMC	23.1	19.4, $p < 0.05$

BMC, bone mineral content; BMD, bone mineral density; F, female; M, male; NS, not significant; pQCT, peripheral quantitative computed tomography.

our methodologies contribute to lack of clarity, but several observations support the importance of adequate dietary calcium and bone development during childhood. Retrospective studies showing a benefit of childhood consumption of milk reducing fracture incidence later in life offer one example. Also, adequate dietary calcium can have a positive interaction with exercise in young children and adolescents. In one study, the presence of both mechanical loading and adequate calcium was required for increased BMD [93]. In other studies, calcium alone increased bone size, an index of greater bone strength [47,88]. Neither the effects of dietary calcium on bone quality nor potential interactions with physical activity were usually considered in the BMD-based RCTs evaluated in the meta-analysis of Winzenberg et al. [94].

One hypothesis to explain the influence of dietary calcium on bone proposed that calcium alters the timing of menarche [95]. Evidence for this possibility came from the RCT of Bonjour et al. [97]. The initial trial was a 1-year intervention of calcium-fortified foods in 7.9-year-old girls that showed a positive effect of calcium on mean BMD changes in six skeletal sites. This cohort was followed through age 16.4 years, which allowed determination of the age of menarche and subsequent changes in bone. Interestingly, girls assigned to the calcium intervention for 1 year began menarche earlier than girls assigned to the placebo group [95]. The extra time of exposure to estrogen resulted in more bone gain in subsequent years even though the calcium intervention was discontinued.

d Dietary Calcium

Calcium intake in most diets is predominantly from dairy products [42]. The particular dairy product distribution varies somewhat with race and ethnicity [96]. The discrepancy between calcium intakes and recommendations becomes a large gap after age 11 years. This is a common problem. Looker [98] compiled calcium recommendations and intakes across the life span for 20 countries. The percentage of adolescents meeting country-specific recommendations was approximately 60% for males and approximately 50% for females. Most children older than age 8 years in the United States have calcium intakes below recommended levels. Only 15% of girls and 23% of boys aged 9–13 years and 13% of girls and 42% of boys aged 14–18 years have calcium intakes above the recommended intakes, according to NHANES data from 2003 to 2006 [99]. However, this is slightly increased in NHANES data from 2001 to 2002, particularly for girls aged 9–13 years and boys aged 14–18 years, which during those years only 6% and 31% met recommended intakes, respectively [100].

It is important to reach children early to establish and maintain dietary habits that will ensure lifelong adequate calcium intakes. There is a fair degree of consistency of calcium intake over time. In a 15-year longitudinal study begun as adolescents, tracking of calcium intake resulted in correlations of $r = 0.43$ for males and $r = 0.38$ for females [101]. Calcium intake is also a marker for intake of other nutrients [42]. This is not true for individuals who consume calcium in the form of supplements.

Calcium absorption from dairy products does not depend on fat content or flavoring. In fact, children who drink flavored milk have higher calcium intakes than non-milk drinkers and have similar total intakes of added sugars in diet and similar BMI as children who drink exclusively white milk [102]. A comparison of calcium bioavailability and the number of servings of various foods required to replace 1 cup of milk or yogurt on the basis of absorbed calcium is given in Table 44.5.

For children 4- to 8-years old, the equivalent of 2.6 cups of milk would be required to meet the RDA, and for children older than 9 years, it would be 4.3 cups. Other foods typically provide the equivalent of at least two-thirds of a cup of milk, so milk is not expected to provide all of the calcium requirements.

e Safety of High Intakes

The UL for calcium is 2500–3000 mg/day [71]. The primary concern for excessive calcium intake from supplements in adults is kidney stones. Interactions with trace mineral absorption are also a concern. Dose–response safety data are not available for children 1- to 18-years old.

2 *Vitamin D*

a Recommendations, Dietary Sources and Intakes, and Status

The RDAs of children for vitamin D are given in Table 44.3. The American Academy of Pediatrics supports these recommendations, but it recommends supplemental vitamin D of 5 μg/day to infants not ingesting fortified formula [104]. The amount of new evidence bearing on inputs for optimal health and safety has been remarkable. However, the strength of the new evidence is largely in adults, and we have much to learn about children. NHANES data from 2001 to 2008 show that 75% of children aged 4- to 18-years old have total vitamin D intakes below the Estimated Average Requirement (EAR) [54], indicating that intakes are falling short of recommendations on a population level.

Vitamin D across the life span and dietary sources of vitamin D are described in Chapter 43, Current Understanding of Vitamin D Metabolism, Nutritional Status, and Role in Disease Prevention. Dietary sources of vitamin D are limited and mostly consumed as fortified foods, especially milk. A summary of studies on vitamin D status in children and adolescents worldwide was compiled by El-Hajj Fuleihan [105]. The range in mean serum 25-hydroxyvitamin D levels (the best status indicator for vitamin D) was large: 13–142 nmol/L. For children at

TABLE 44.5 Comparing Sources for Absorbable Calcium

Source	Serving Size (g)	Calcium Content (mg/serving)	Estimated Absorption Efficiency (%)	Food Amount to Equal Calcium in 1 C Milk
Milk	240	300	32.1	1.0 C
Beans, red	172	40.5	24.4	4.8 C
Beans, white	110	113	21.8	2.0 C
Bok choy	85	79	53.8	1.2 C
Broccoli	71	35	61.3	2.3 C
Cheddar cheese	42	303	32.1	1.5 oz.
Cheese food	42	241	32.1	1.8 oz.
Chinese cabbage flower leaves	85	239	39.6	0.5 C
Chinese mustard green	85	212	40.2	0.6 C
Chinese spinach	85	347	8.36	1.7 C
Kale	85	61	49.3	1.6 C
Orange juice with calcium citrate maleate	240	300	36.3	0.9 C
Soy milk with calcium phosphate	240	300	24.0	1.3 C
Spinach	85	115	5.1	8.1 C
Tofu, calcium set	126	258	31.0	0.6 C
Whole wheat bread	28	20	82.0	5.8 slices
Wheat bran cereal	28	20	38.0	12.8 oz.
Yogurt	240	300	21.1	1.0 C

Source: Taken from C.M. Weaver, R.P. Heaney, Food sources, supplements and bioavailability, in: C.M. Weaver, R.P. Heaney (Eds.), Calcium in Human Health, Humana Press, Totowa, NJ, 2006, 129–142 [103], with kind permission of Springer Science and Business Media.

risk for vitamin D deficiency, recommended intakes are 600–1000 IU/day.

b Evidence for a Relationship to Bone

Vitamin D deficiency in young children has long been associated with rickets. An intake of 2.5 μg (100 IU)/day is thought to be adequate to prevent rickets [105,106]. This disorder is described in Chapter 43, Current Understanding of Vitamin D Metabolism, Nutritional Status, and Role in Disease Prevention.

The vitamin D–parathyroid hormone (PTH) homeostatic regulatory axis helps regulate serum calcium levels in response to dietary calcium. Under conditions of low calcium intake, serum calcium levels fall, PTH is released, and vitamin D is converted to its active form, 1,25-dihydroxyvitamin D. Serum calcium levels rise as intestinal calcium absorption is upregulated; renal reabsorption of calcium increases, which conserves urinary excretion of calcium; and bone resorption increases. Interestingly, improving vitamin D status with supplementation of 1000 IU (25 μg) vitamin D_3 per day for 4 weeks resulted in a significant decrease in fractional calcium absorption in adolescents [107]. Apparently, vitamin D status was adequate at 4.5 nmol serum 25(OH)D and/or calcium intake of approximately 1000 mg/day was sufficiently high without supplementation to optimize fractional calcium absorption. Similarly, a 12-week dose–response RCT of vitamin D_3 supplementation, ranging from 400 to 4000 IU/day in children in early puberty, found no effect of supplementation at any level on fractional calcium absorption [108]. This was despite significant increases in serum 25(OH)D over the 12-week study. However, mean baseline 25(OH)D levels were sufficient at 70 nmol/L. Response to vitamin D levels used in the RCTs would only have the potential to be effective if doses were adequate and beginning vitamin D status of the subjects was sufficiently inadequate.

The NOF position paper rated the level of evidence as grade B for vitamin D and development of peak bone mass [1]. The seven RCTs relating vitamin D supplementation and bone (Table 44.6) are quite varied among the

TABLE 44.6 Differences in Mean Changes in BMC and BMD in Vitamin D Versus Placebo in RCTs in Children

Source	Ref. No.	Subj. No.	Age (years)	Sex	Race/ Location	Length Study (months)	Vitamin D Supplement (μg/day)[a]	Site	Measure	Group Mean Increase (% Unless Otherwise Indicated)	
										Treatment (T)	Placebo (P)
Cheng et al. (2005)	[89]	195	10–12	F	White, Finland	24	5	Whole body	BMC	34.7	35.0, NS
								Femoral neck	BMC	24.0	22.4, NS
								Total femur	BMC	33.6	33.6, NS
								Spine	BMC	46.9	47.0, NS
								Tibia cortical thickness	pQCT	31.7	31.1, NS
El-Hajj Fuleihan et al. (2006)	[109]	179	10–17	F	White, Lebanese	24	5	Total body	BMC	11.3	8.7, NS
								Total hip	BMC	11.2	7.8, NS
								Total hip	Area	4.0	2.4, $p = 0.05$
								Femoral neck	BMC	4.4	3.9, NS
								Femoral neck	Area	0.03	0.7, NS
								Trochanter	BMC	13.6	9.4, NS
								Trochanter	Area	6.8	4.7, NS
								Total body	BMC	12.0	8.7, NS
								Total hip	BMC	12.8	7.8, $p = 0.005$
								Total hip	Area	5.7	2.4, $p = 0.001$
								Femoral neck	BMC	5.2	3.9, NS
								Femoral neck	Area	0.8	0.7, NS
								Trochanter	BMC	14.2	9.4, NS
								Trochanter	Area	7.8	4.7, NS
Viljakainen et al. (2006)	[110]	212	11.4 ± 0.4	F	White, Finland	12	5	Femur	BMC	14.3 (T vs P), $p = 0.012$	
								Lumbar spine	BMC	NS	
							10	Femur	BMC	17.2 (T vs P), $p = 0.012$	
								Lumbar spine	BMC	12.5 (T vs P), $p = 0.039$	

(*Continued*)

TABLE 44.6 (Continued)

Source	Ref. No.	Subj. No.	Age (years)	Sex	Race/ Location	Length Study (months)	Vitamin D Supplement (μg/day)[a]	Site	Measure	Group Mean Increase (% Unless Otherwise Indicated)	
										Treatment (T)	Placebo (P)
Khadilkar et al. (2010)	[111]	50	14–15	F	India	12	7500	Total body	BMC	10.1 vs 8.2	NS
									Area	5.1 vs 3.6	NS
Mølgaard et al. (2010)	[112]	225	10–11	F	Denmark	12	5	Total body	BMC (g)	245 vs 241	NS
									Area (cm^2)	189 vs 188	NS
								Lumbar spine	BMC (g)	8.1 vs 8.2	NS
									Area (cm^2)	5.5 vs 5.6	NS
							10	Total body	BMC (g)	249 vs 241	NS
									Area (cm^2)	195 vs 188	NS
								Lumbar spine	BMC (g)	7.4 vs 8.2	NS
									Area (cm^2)	4.9 vs 5.6	0.04
Ward et al. (2010)	[113]	73	12–14	F	Multiethnic South Asia	12	4–3750	Lumbar spine	BMC (g)	0.52 vs 0.57	NS
									Area (cm^2)	0.2 vs 0.15	NS
								Tibia 4%	Total BMD (mg/cm^3)	10.5 vs 9.79	NS
									CSA (mm^2)	−1.19 vs −3.28	NS
Al-Shaar et al. (2013)	[114]	338	10–17	F	Beirut, Lebanon	12	Weekly 35	Narrow neck	CSA	8.3 vs 8.2	NS
									Outer diameter	0.8 vs 2.8	0.02
									Buckling ratio	−6.5 vs −2.0	0.05
							Weekly 350	Narrow neck	CSA	8.1 vs 8.2	NS
									Outer diameter	2.0 vs 2.8	
									Buckling ratio	−4.2 vs −2.0	

[a]*Analysis included only those >80% compliant.*
BMC, bone mineral content; F, female; NS, not significant; pQCT, peripheral quantitative computed tomography.

studies. As with calcium absorption, vitamin D and calcium intakes would be interdependent.

One RCT of two doses of vitamin D equivalent to 200 and 2000 IU/day in 10- to 17-year-old Lebanese girls was able to improve vitamin D status on the higher dose and improve total hip BMC and area but not other bone measures [109]. Dietary calcium intakes were low in that study. Lean mass was increased with vitamin D supplementation. Subjects in both the Cheng et al. [89] study and the Viljakainen et al. [110] study had adequate calcium together with vitamin D.

c Safety of High Doses

Dose–response studies of longer duration than have been reported are needed to truly determine ULs of safety studies in which risk intoxication is unmentioned. The current ULs are given in Table 44.3.

Safety is assessed by observing no change in serum or urinary calcium levels. The 5-μg dose of vitamin D was ineffective in the Cheng et al. [89] study but effective for the femur in the study by Viljakainen et al. [110], but only when a compliance-based analysis was used. That study showed a dose–response change in vitamin D. Single oral doses of vitamin D have been given with no change in mean serum or urinary Ca:Cr ratios [110].

In the study by El-Hajj Fuleihan et al. [109], adolescents given 14,000 IU (350 μg/week) of vitamin D_3 for 1 year showed no change in mean serum calcium level. This is equivalent on a daily dose to the UL. We do not know the safety of much higher doses in children.

3 Other Nutrients

Although many other nutrients are necessary for growing bone, there is little evidence that current intakes compromise development of peak bone mass for most individuals. Two minerals besides calcium that comprise a substantial protein of bone mineral are phosphorus and magnesium. Bone mineral is calcium phosphate, but 60% of the magnesium in the body resides in bone. RDAs and ULs for these nutrients for children are given in Table 44.3. The dietary Ca:P ratio affects bone mineralization and turnover through intestinal calcium and phosphorus transports [115]. Phosphorus is clearly essential for bone acquisition, but deficiency has not been a concern for children. Excessive intakes of phosphorus in soft drinks have been a concern. However, as discussed previously, the negative association of soft drink consumption and bone in girls is likely due to displacement of milk as a beverage.

Magnesium deficiency disrupts bone accretion. Rats fed 50% of their requirement for magnesium have structural changes that lead to reduced bone volume [116]. Obtaining recommended intakes of magnesium is not of concern for children. Iron deficiency also has a detrimental effect on bone morphology in growing animals that is exacerbated by calcium deficiency [117].

V CONCLUSION

Making wise nutritional choices during growth is a window of opportunity to build optimal peak bone mass to reduce risk of fracture later in life. If the opportunity is neglected, the consequence can be fracture. During infancy, it is not difficult to meet requirements through breast-feeding or infant formulas. The other accelerated growth period, puberty, is much more difficult. Diets vary widely in nutrient sufficiency; peers may have more influence than caregivers. Fracture incidence in childhood is highest during the pubertal growth spurt when bones elongate before they consolidate and BMD is lower. The incidence of pubertal fractures is increasing, possibly related to the increase in obesity coupled with a decline in consumption of milk as the beverage of choice. Diet patterns may be as important in building strong bones as adequacy of individual nutrients. The *Dietary Guidelines* offer a good plan. Supplements may be useful for some individuals and in some conditions.

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Chapter 45

Osteoporosis in Adults

Robert Marcus
Stanford University, Stanford, CA, United States

I INTRODUCTION

Osteoporosis is a global skeletal disorder of decreased bone strength in which the only important consequence is an increased risk for fracture with minimal trauma. The term *porosis* means "spongelike," which aptly describes the appearance of that portion of the skeleton, the trabecular skeleton, which is most afflicted with this disease (Fig. 45.1). Bone strength is a complex function integrating bone mineral density (BMD) and bone quality. BMD is the concept most familiar to the public, to patients, and to physicians, but it is only one determinant of bone strength, all other contributions that are not captured by BMD measurement come under the rubric of "bone quality." The term *quality* should more properly be considered as the plural *qualities* because it encompasses a wide variety of characteristics that are enumerated later in this chapter. Osteoporotic, or fragility, fractures traditionally have been grouped according to their location, either in the spine (vertebral) or nonspine, the latter including those of the distal radius (Colles' fractures) and the proximal femur (hip fracture). It is important to understand that because of its global nature, osteoporosis imposes an increased risk for virtually all fractures in affected individuals.

Already more common than any other generalized skeletal condition, osteoporosis continues to increase in prevalence. Based on data from the National Health and Nutrition Examination Survey III (NHANES) and from the 2000 National Census, the National Osteoporosis Foundation estimated that, in 2002, 20% of postmenopausal white women in the United States had osteoporosis, and an additional 52% had low bone density at the hip [1]. In the whole population, approximately 8 million have osteoporosis, of whom approximately 1.5 million will fracture each year. One out of every two white women will experience an osteoporotic fracture at some point in her lifetime [1−3].

Although men have a lower prevalence of osteoporosis than women, perhaps 25% as great, fragility fractures certainly occur in men, and some of these, particularly at the hip, carry a less favorable prognosis in men than for women. The most common osteoporotic fractures occur as compression deformities of the thoracic or lumbar spine. Although two-thirds of these do not acutely produce symptoms, they must not be viewed in any sense as benign events. Even mild compression fractures aggravate by four- or fivefold the short-term risk for subsequent fractures. Approximately one-third of vertebral fractures produce symptoms when they occur and are referred to as "clinical" vertebral fractures. These are more likely to be of moderate or severe degree in deformity and are the fractures most likely to result in long-term pain, deformity, and disability. In the Study of Osteoporotic Fractures (SOF), a long-term observational study of thousands of older women, clinical vertebral fractures were associated with an eightfold excess of mortality similar to that observed with hip fracture [4]. The incidence of vertebral fractures in women begins to rise early in the sixth decade, corresponding in time to the menopausal loss of endogenous estrogen. The incidence continues to increase in succeeding decades (Fig. 45.2) [3].

At nonvertebral sites, forearm fractures, particularly at the distal radius, also increase during the sixth decade but stabilize thereafter, at which time the incidence of hip fracture begins exponentially to increase [3]. Both forearm and hip fractures result directly from a fall. Whether an individual fractures the arm or the hip reflects the manner of falling. Younger women with normal locomotion generally fall while walking and break a fall by arm extension. Older and more frail women often fall while transferring from a seated to a standing position. If they

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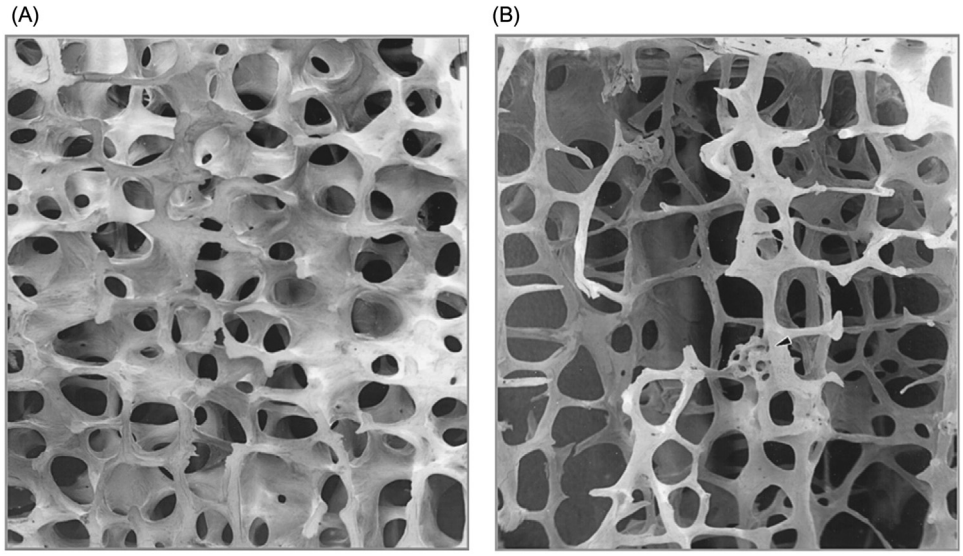

FIGURE 45.1 (A) Normal trabecular bone. Note the highly interconnected vertical and horizontal bars, fairly homogeneous size and shape of holes, and platelike appearance of many of the trabecular units. (B) Osteoporotic bone. Note substantial reduction in the amount of bone substance per unit volume compared to normal bone (A). Note the narrow rodlike appearance of vertical trabeculae compared to the normal platelike structures. Note the wide variation in the size of holes throughout the trabecular structure. In many regions, trabecular struts are hanging in space without connection to neighboring structures. *Courtesy Dr. David Dempster. Copyright David Dempster.*

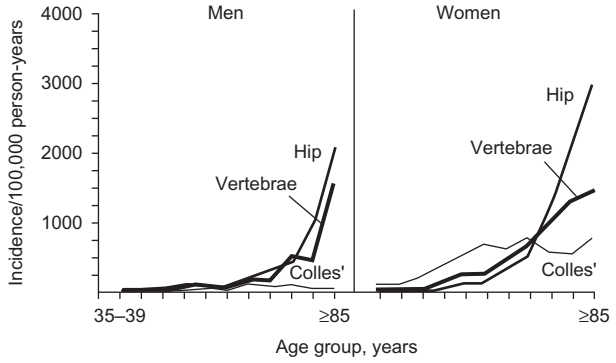

FIGURE 45.2 Age-specific incidence rates for hip, vertebral, and distal forearm fractures in men and women. *Data derived from the population of Rochester, Minnesota. From C. Cooper, L.J. Melton, III, Epidemiology of osteoporosis, Trends Endocrinol. Metab. 3 (1992) 224–229.*

fail to elevate their centers of gravity sufficiently to support an upright posture, they fall backwards or to the side, directly impacting the femoral greater trochanter and possibly leading to hip fracture [5,6].

This chapter focuses on the characteristics of a healthy skeleton, the underlying pathophysiology of osteoporosis, the characteristics of osteoporotic bone, approaches to conserving bone throughout adult life, and therapeutic approaches to treating skeletal fragility. A comprehensive discussion of the various pharmacologic agents available for patient management lies outside the scope of this chapter, and the critical role of bone acquisition during years of growth through adolescence appears in the companion chapter in this volume on childhood bone development (Chapter 44: Osteoporosis: The Early Years) which the reader is strongly urged to consult.

II THE SKELETON

Bone is a complex cellular tissue that contains, by weight, approximately 30% organic constituents and 70% mineral. The most abundant protein in the organic compartment is type I collagen, a fibrillar structure consisting of three interweaving strands—normally two strands of alpha-1 collagen and one strand of alpha-2 collagen. Collagen represents 98% of the organic phase of bone, and various noncollagen proteins account for the remainder [7].

The mineral phase of bone is approximately 95% hydroxyapatite, a highly organized crystal of calcium and phosphorus. Other minerals normally found in bone mineral include sodium (indeed, approximately 30% of total body sodium can be stored in bone crystal), magnesium, and fluoride. Incorporation of fluoride and strontium into bone crystal is of particular relevance because these compounds have seen use as therapy for osteoporosis.

A Bone Cells

The processes of bone formation and breakdown (resorption) require cellular activity. Three major cell types reside in bone and conduct these processes: osteoblasts, osteocytes, and osteoclasts.

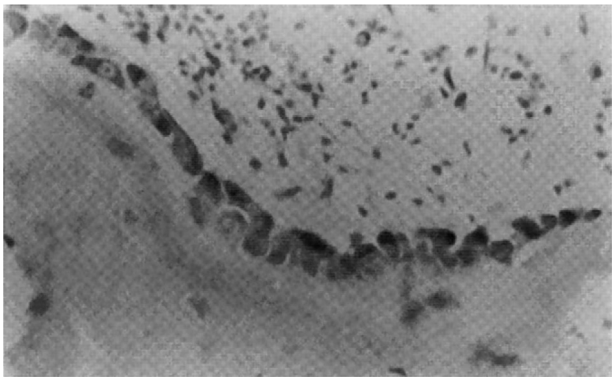

FIGURE 45.3 Low-power view of osteoblasts lining the bone surface. *From C.A. Lee, T.A. Einhorn, The bone organ system, form and function, in: R. Marcus, D. Feldman, J. Kelsey, Osteoporosis, Academic Press, San Diego, CA, 2001, 3–20 [7], with permission.*

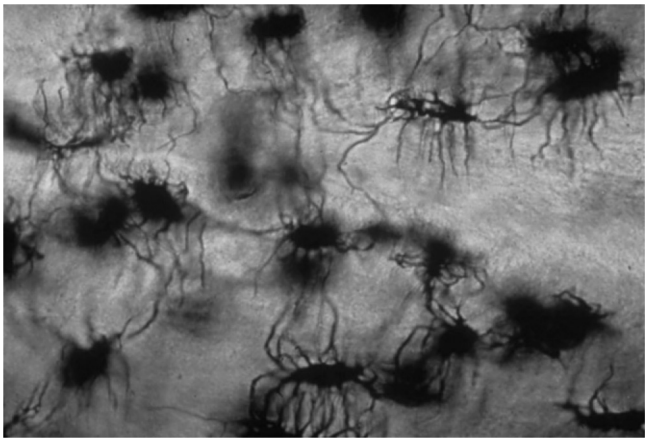

FIGURE 45.4 Osteocytes occupying individual lacunae with extensive canalicular interconnections. *From C.A. Lee, T.A. Einhorn, The bone organ system, form and function, in: R. Marcus, D. Feldman, J. Kelsey, Osteoporosis, Academic Press, San Diego, CA, 2001, 3–20 [7], with permission.*

FIGURE 45.5 Low-power view of osteoclasts occupying resorption lacunae. *From C.A. Lee, T.A. Einhorn, The bone organ system, form and function, in: R. Marcus, D. Feldman, J. Kelsey, Osteoporosis, Academic Press, San Diego, CA, 2001, 3–20 [7], with permission.*

Osteoblasts are the primary bone-forming cells. They derive from stem cells in the bone marrow stroma. These stem cells are pluripotential, having the capacity to develop along multiple lineages, including fibroblasts, hematopoietic cells, myocytes, adipocytes, chondrocytes, and osteoblasts. During linear growth, osteoblasts invade a temporary cartilaginous template to form primary lamellar bone. During remodeling (see later discussion), a wave of osteoblast precursors migrates to the base of a resorption cavity, acquires the characteristics of mature osteoblasts, and lays down new bone (Fig. 45.3).

Osteocytes are osteoblasts that have become embedded within their own secreted matrix. Each osteocyte sits in its individual hole, or *lacuna*, connected to one another throughout the bone matrix by a highly developed network of channels, or *canaliculi*. Osteocytes appear to be the monitors and responders to a bone's mechanical environment (Fig. 45.4).

Osteoclasts are multinucleated giant cells of macrophage lineage. They undertake the enzymatic destruction of bone during the resorption phase of remodeling (see later discussion). During this process, osteoclasts form a seal at the bone surface with the aid of anchoring proteins called integrins whose receptors exist in the bone matrix. This seal creates a sequestered region underneath the osteoclast into which hydrogen ion is secreted using a carbonic anhydrase-dependent pump and resulting in a highly acidic local environment. In addition, the osteoclast secretes a variety of hydrolytic enzymes, such as cathepsins, which hydrolyze bone matrix (Fig. 45.5).

A fourth cell is also observed in bone. So-called *lining cells* are seen as a syncytial layer of dormant cells that covers bone surfaces. This group of cells is thought to serve a surveillance function that responds to microscopic damage by locally stimulating new remodeling activity. Lining cells also originate from osteoblasts and, although dormant, retain the capacity in certain circumstances to convert into functional osteoblasts and lay down new bone. This appears to be one mechanism through which administration of parathyroid hormone (PTH) achieves a rapid increase in bone formation (see section VB on Pharmacologic therapeutics).

Eighty percent of the adult skeleton consists of compact bone. This is referred to as the *cortical* or *appendicular* skeleton and comprises mostly the long bones as well as the outer shells of the central, or *axial*, skeleton, which includes the spine, sternum, pelvis, and the ends (metaphyses) of long bones. The axial skeleton has a heavy complement (perhaps 40% by weight or 80% by surface area) of a honeycomb-like series of vertical and horizontal bars, or *trabeculae*, and is therefore frequently called

trabecular bone (in orthopedics this may also be called *cancellous* bone). In adults, the trabecular bone of the spine and pelvis constitutes the primary residence of red bone marrow. Because the cells responsible for conducting the processes underlying adult bone loss originate in the bone marrow, and because these processes occur on the surfaces of bone, it should be no surprise that trabecular bone, with its rich complement of bone marrow and extensive surface area, should be the bone compartment that experiences the earliest and most rapid loss of bone with aging.

At any time during adult life, the amount of bone contained within the skeleton consists of that bone which was present at the end of growth, the so-called "peak bone mass," minus that which has been lost. One frequently encounters patients who report being told, following a bone density test, that they have "lost 30% of their skeleton." The problem with such conclusions is that one simply cannot determine from a single BMD measurement whether a deficit in bone mineral reflects bone loss or failure to achieve the peak bone mass that might have been predicted for that individual. In fact, the majority of young adults with low bone mass have not lost bone at all but, rather, have age-related deficits related to poor acquisition of peak bone mass. (See chapter 44 by Weaver on childhood bone acquisition in this volume.)

B Physiologic Roles of the Skeleton

During vertebrate evolution, the skeleton acquired two fundamental but not necessarily compatible functions. By virtue of its dense mineralization, bone provides the structural rigidity necessary to withstand the effect of gravity and support terrestrial locomotion. By adapting to region-specific differences in its mechanical environment, denser, stronger bone exists where it is needed without requiring a universal increase in skeletal weight to the point that mobility is jeopardized.

Bone also constitutes the *primary* repository in the body for calcium. Indeed, 99.5% of body calcium is contained within bone and can be mobilized to support the extracellular calcium concentration at times of need. For the great majority of vertebrates, the calcium environment is extremely high, reflecting its very high concentration in ocean water ($\sim$400 mg/L). Facing the threat of calcium toxicity, ocean fish must be able to eliminate excess calcium from their bodies, which they accomplish through a calcium-dependent ATPase system in the gills. Progression of vertebrates onto land, with freshwater far more dilute in calcium, required mechanisms to promote calcium extraction from the environment and to conserve it within the body. PTH, the peptide secretory product of the parathyroid glands (first appearing in amphibians), serves this role. In response to minute-to-minute relatively mild reductions in extracellular calcium concentration, such as during the hours following a meal, PTH stimulates the kidney to conserve calcium by regulating renal tubular reabsorption efficiency. When calcium deficits become sustained or severe, such as in the face of chronically inadequate dietary calcium, PTH stimulates the renal production of $1,25(OH)_2$ vitamin D (calcitriol), the potent hormonal form of the parent vitamin, which in turn enhances intestinal calcium absorption. In this setting, PTH also stimulates bone remodeling by initiating the formation of new remodeling units and resulting in delivery of calcium from the skeleton to the extracellular environment. Together, these actions restore plasma calcium concentrations to their normal level [8]. It must be understood that PTH action on the skeleton does not selectively remove calcium from the skeleton but is accomplished by an increase in bone remodeling (see later discussion) so that the release of mineral to the plasma compartment is accompanied by a net loss of bone.

C Remodeling: The Key to Understanding Age-Related Bone Loss

Many mammals, primates, and humans maintain skeletal integrity through a continuous process of breakdown and renewal known as *remodeling*. This process occurs lifelong, although during childhood and adolescence it is overshadowed by the events of linear growth (modeling). Once growth centers have fused and skeletal maturation is complete, remodeling becomes the dominant—and indeed, with rare exception, the only—mechanism through which bone is added to or removed from the skeleton. Each remodeling event is carried out by discrete bone multicellular units and consists of an initial phase of bone resorption that is coupled to a longer phase of bone formation (Fig. 45.6). These are initiated when cells of macrophage lineage come from the bone marrow to points on the bone surface and fuse into multinucleated osteoclasts that dig into and remove bone. The cavity thus created reaches a depth of 60 μm within 6−8 weeks. In this manner, both mineral and matrix constituents are returned to the circulating extracellular fluid. Released from the resorbed matrix is a rich assortment of cytokines and growth factors that then attract into the base of the cavity a wave of osteoblast precursor cells from the marrow stroma. These transform into functional osteoblasts and begin to lay down new bone matrix. Once the new bone reaches a thickness of approximately 20 μm, it begins to accumulate mineral. By the end of approximately 6 months, bone formation is complete and the bone is restored almost to its basal state. However, like many biological processes, bone remodeling is not 100% efficient; that is, the amount of new bone formed does not

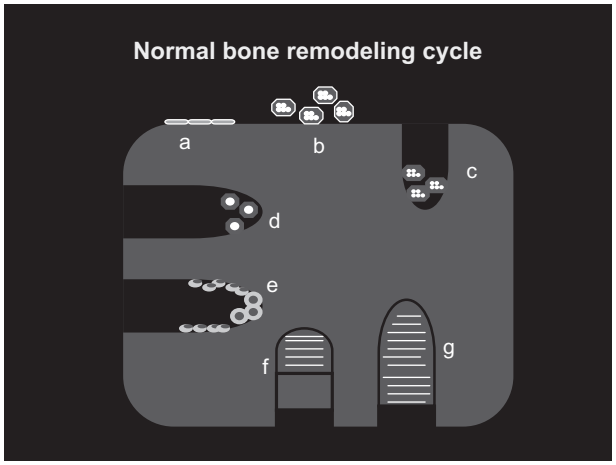

FIGURE 45.6 The bone remodeling cycle. This drawing represents a region of trabecular bone. All remodeling events occur on the bone surface. (a) 90% of bone surface is generally covered by thin layer of dormant lining cells. (b) Coalescence of osteoclast precursors at a site on the bone surface with creation of multinucleated osteoclasts. (c) Osteoclasts remove a divot of bone, reaching 60 μm in depth by 6–8 weeks. (d) Soluble factors released by osteoclastic resorption recruit a new wave of cell proliferation (preosteoblasts) into the base of the resorption cavity. (e) Preosteoblasts acquire the osteoblast phenotype. (f) Osteoblasts secrete new bone matrix, which begins to acquire mineral after a thickness of approximately 20 μm is achieved. (g) New mineralized bone almost fully replaces resorbed bone by approximately 6 months. Small deficits are left, reflecting remodeling inefficiency and accounting for the process of age-related bone loss. *Copyright Robert Marcus.*

completely make up for the older bone removed, so a small bone deficit remains as a consequence of each remodeling event, called the "remodeling imbalance." The cumulative effect of the hundreds of thousands of remodeling units in play at any one time is the readily observable phenomenon of age-related bone loss. Consequently, anything that promotes an overall increase in whole body bone remodeling aggravates the rate of bone loss and, by contrast, interventions that slow remodeling constrain bone loss. This forms the basis for using drugs that slow remodeling as a mainstay of osteoporosis treatment, as discussed later in this chapter.

Another word should be said concerning matrix mineralization during the bone formation phase of remodeling. Mineral is rapidly laid down for the initial several weeks but thereafter changes to a slow, linear rate. Mineral is never fully saturated in the bone, so it continues to accumulate as long as that particular unit of bone survives. The only thing to terminate this process would be the initiation of a new wave of osteoclastic resorption to clear out this region of older bone. Thus, if overall remodeling slows, bone survives longer and becomes more densely mineralized. The consequences of this finding are also discussed later.

If, as described, bone remodeling leads to loss (and presumably weakening) of bone, one may ask why it has evolved and what its purpose may be. It appears that the cardinal role of remodeling is to serve a scavenger function, through which fatigued or damaged bone is cleared away and replaced (albeit at the long-term price of gradual bone loss). In addition, remodeling is the means by which PTH restores normocalcemia in response to hypocalcemia.

D Intercellular Communication Among Bone Cells: Triggers and Constraints on Remodeling

Because initiation of each remodeling event begins with delivery of osteoclasts or their precursors to the bone surface, it is important to have passing familiarity with the signals that control this initial event [9,10]. The key cell for controlling osteoclast production is the osteoblast. This bone-forming cell elaborates two distinct proteins, a stimulator and a repressor, that regulate osteoclast production. The osteoclast stimulator was initially called "osteoclast differentiation factor," but now that its primary target is known, it has been given the less transparent name of RANK-ligand. RANK is an abbreviation for the "receptor that activates NF-kappa β" (a gene present in osteoclasts and other cells of macrophage origin). Under stimulation by agents known to increase bone remodeling (e.g., PTH and L-thyroxine), the osteoblast synthesizes and extrudes RANK-ligand that binds to RANK located on osteoclast precursors, leading to production of new osteoclasts. The repressor molecule, also produced by osteoblasts, is a protein called osteoprotegerin (OPG). Production of this protein increases when the osteoblast binds agents that inhibit remodeling, such as estrogen. OPG exhibits high affinity for RANK-ligand and therefore acts as a false receptor, neutralizing the effect of any RANK-ligand with which it comes into contact.

The RANK–RANK-ligand–OPG complex acts in a push–pull manner to regulate osteoclast production and hence controls the rate of bone remodeling. When stimuli favor greater remodeling, RANK-ligand production increases, OPG decreases, and RANK is activated. When remodeling is suppressed, RANK-ligand decreases, OPG increases, and RANK is constrained. Interference with RANK-ligand function is the basis of a recently developed therapy for osteoporosis (see Section V.B.1.f: Denosumab).

III ADULT BONE MAINTENANCE

For many years, scientific inquiry into the basis of adult bone loss and development of osteoporosis was highly

parochial. Nutrition scientists focused on the diet, exercise physiologists and mechanical engineers focused on physical activity and the mechanical environment, and physicians whose responsibility was to care for patients with osteoporosis focused largely on menopausal estrogen loss. It is now abundantly clear that acquisition and maintenance of a healthy skeleton is far more complex than can be explained by any of these individual spheres. One needs to view the skeleton as subject to diverse influences throughout life so that bone status at any particular time is the result of a stochastic process by which each individual insult or event over a lifetime has made its independent contribution.

A Major Influences on Age-Related Bone Loss

Successful bone maintenance requires continued attention to the same "hygienic" factors that influenced bone acquisition: physical activity, diet, and reproductive status. Bone maintenance requires sufficiency in all areas, and the others do not compensate deficiency in one. For example, amenorrheic athletes lose bone despite frequent high-intensity physical activity and supplemental calcium intake [11,12]. Successful bone maintenance is also jeopardized by known toxic exposures such as smoking, alcohol excess, immobility, systemic illnesses, and many medications.

1 Habitual Physical Activity

The skeleton's mechanical function was referred to previously. To accomplish this role in a manner that optimizes bone strength while at the same time not unduly increasing its weight, bones accommodate the loads imposed on them by undergoing alterations in mass, in external geometry, and in internal microarchitecture. The first enunciation of this principle is credited to the German scientist Julius Wolff as "Wolff's law" [13]. As a consequence of such adaptation, steady-state bone mass reflects its mechanical environment, a concept that applies when comparing bone mass among individuals, different bones within an individual, and even different regions within a single bone. A substantial body of research has addressed this concept. These studies are of two general types: (1) comparisons of bone mass of athletes to that of sedentary controls and (2) descriptions of associations between level of physical activity and bone mass within a general population. The first type of study generally considers only very active or sedentary individuals, and hence extreme differences in activity are represented. In the latter case, a broader range of physical activity is examined.

Considerable evidence indicates that elite athletes and chronic exercisers have higher BMD than age-matched, nonexercising subjects—a finding, not surprisingly, that applies primarily to sites that undergo loading during the exercise (reviewed in Ref. [14]). Activities associated with high load magnitude at low number of repetitions (cycles) are associated with substantial increases in bone mass. For example, world-class and recreational weight lifters have 10−35% greater lumbar spine BMD than sedentary age-matched controls. Comparing dominant to nondominant limbs in athletes whose sport involves unilateral loading represents a special case. For example, increased BMD in the playing compared to the nonplaying arm of tennis players has been repeatedly observed. By contrast, swimming, a buoyant activity not associated with counteracting the effect of gravity, does not appear to increase BMD. In one study of elite university athletes, swimmers actually had lower bone mass than gymnasts or nonathletic controls, despite increased muscle bulk and regular weight training [15]. Young athletes who spend more than 20 hours each week in a buoyant environment for many years may simply not experience sufficient gravitational stress to promote fully the expected degree of bone acquisition.

These comparisons of athletes and control subjects must be interpreted with caution. Because no measurements of bone mass are made prior to initiating the exercise program, a causal relationship between exercise and bone cannot be proven. It may be that individuals with higher bone density are more apt to succeed in athletics, and therefore they enter the "athlete" and chronic exercising groups. Conversely, elite swimmers may have excelled in buoyant activity because of a lighter skeleton. In many studies, important characteristics of the matched controls have been overlooked. Factors such as menstrual status, nutrient intake, and use of tobacco or alcohol may have confounded the results. Finally, skeletal status is most frequently expressed as the areal BMD (g/cm^2), a term that overestimates BMD in persons with large bones and underestimates it in smaller people. Thus, if exercisers and controls are not well matched for height, conclusions based on BMD may be spurious.

With respect to the impact of habitual physical activity within the general population, many studies now point to a significant skeletal effect of physical activity on the acquisition of bone during the second and third decades. The situation is less clear for moderately active adults, in whom no consistent relationship between current activity level and bone mass has been established. In our own work, strong relationships between estimates of daily energy expenditure and BMD were completely negated by normalizing the data for body weight or lean mass [14]. Several reports document positive relationships between lifelong physical activity and bone status.

Thus, cross-sectional studies generally support the notion that elite athletes and chronic exercisers have

increased BMD, the magnitude of this difference likely depending on the type and intensity of exercise, age, sex, and hormonal status. However, data concerning moderate physical activity remain uncertain.

One approach to eliminating the selection bias of cross-sectional studies is a randomized controlled trial in which exercise is the intervention. A number of properly controlled studies of this sort have been reported. Although most indicate a positive effect of imposed exercise on BMD, the magnitude of response has been very disappointing to those who anticipated the large differences observed in cross-sectional studies. Rarely do the increases in BMD exceed 2% after 1 or 2 years of rigorous training, regardless of the type of exercise used (endurance vs resistance training) [16–19].

Understanding the meager response to exercise interventions is related to the fact that skeletal response to mechanical loads is curvilinear in nature. Complete immobilization, as seen with high-level spinal cord injury, leads rapidly to devastating bone loss, with deficits approaching 30–40% over several months. By contrast, imposition of even substantial training regimens on normally ambulatory people or animals increases bone mass by only a few percent over a similar period. This is illustrated in Fig. 45.7, in which the effect of walking on bone mass is schematized. As an individual goes from immobility to full ambulation, duration of time spent walking becomes progressively less efficient for increasing bone mass. A person who habitually walks 6 hours each day might require another 4–6 hours just to add a few more percent BMD. On the other hand, adding a more rigorous stimulus, such as high-impact loading, for even a few cycles would increase the response slope.

The worst thing that can happen to the skeleton is to be immobilized. For maintaining bone mass during adult life, a certain degree of daily weight bearing is required, and the vast majority of even sedentary individuals achieve this. Small increments in BMD can be achieved by increasing one's daily exercise schedule, but, as a consequence of Wolff's law, these will remain only so long as the added activity continues. For most individuals, particularly if they are elderly or frail, walking provides the soundest and most prudent physical activity for skeletal maintenance.

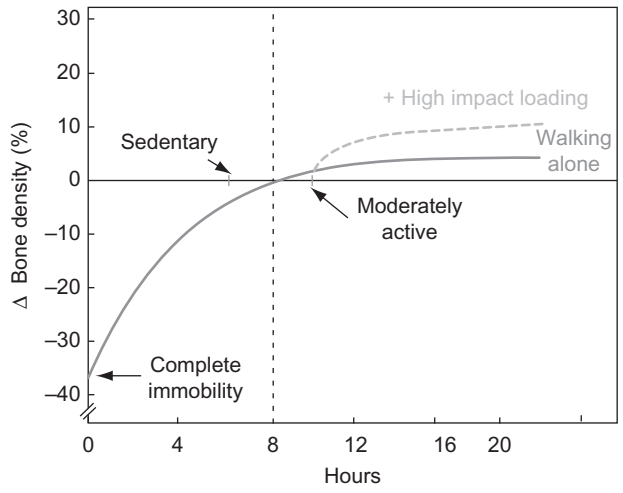

FIGURE 45.7 The curvilinear nature of skeletal response to mechanical loading. *Copyright Robert Marcus.*

2 Nutrition

a Energy

Reflecting the major influence of weight-bearing activity on bone, bone mass is strongly related to body weight, so it should be no surprise that severe deficits in bone occur in states of profound malnutrition. Although frank starvation is extremely rare in developed societies, bone deficits are frequently encountered in medical conditions associated with extremely low body mass, such as anorexia nervosa, as well as various forms of intestinal malabsorption and cachexia. It may be difficult to assign responsibility for bone deficits in such patients to any specific nutrient because patients generally show profound reductions in the consumption of many nutrients. In teenagers with anorexia nervosa, skeletal deficits appear very early, and their magnitude is exacerbated because bone is lost at a time when other girls of similar age are gaining bone at an accelerated rate. Bachrach et al. [20] observed that whole body bone mass correlates linearly with body mass in normal teens, and that the bone mass of girls with anorexia nervosa lies exactly on this curve. In other words, skeletal deficits in young girls with anorexia nervosa primarily reflect their body mass. When these girls were observed over time, weight rehabilitation (a gain of at least 5 kg) was associated with a gain in bone mass.

The next section deals specifically with a group of skeletally important nutrients and nutrient groups. The reader might find it helpful once having read this material to consult the recently developed U.S. Government website ChooseMyPlate (www.choosemyplate.gov) in which food patterns were designed to meet the bone nutrient requirements.

b Calcium

The concept that osteoporosis is a disease of calcium deficiency was proposed more than a century ago, although a central role for calcium intake did not emerge into the scientific mainstream for many years. This largely reflected the overpowering influence of Professor Fuller Albright, who conceived of osteoporosis as a deficiency of bone matrix due to osteoblast failure, usually as a consequence of menopausal loss of estrogen [21]. Intensification of interest in calcium occurred with the publication of three

independent reports. The first, by Matkovic et al. [22], demonstrated a difference in hip fracture incidence in two different regions of Croatia that were demographically very similar except for a substantial difference in the calcium content of local water supplies. Second was the publication of NHANES II, which showed that habitual calcium intakes of American girls and women failed to meet recommended dietary allowances (RDAs) as early as age 11 years [23]. Third was a landmark paper by Chapuy et al. [24] that clearly demonstrated the ability of supplemental calcium and vitamin D to reduce the incidence of hip fracture in a highly vulnerable population.

When one considers that the skeleton is the repository for more than 99% of total body calcium, it is inconceivable that individuals whose intakes are below the amount necessary to maintain whole body calcium balance could be in negative balance without losing bone. Calcium may be considered a "threshold nutrient," which means that below a critical value some physiological function (e.g., calcium balance) is dependent on intake, but at intakes above that value no further impact accrues. Because the mineral demands for growth, early adult, and older adult maintenance differ substantially, the "threshold" value and therefore the intake requirements also differ by age [25,26]. Table 45.1 shows that recommendations for calcium intake have increased substantially over time from previous RDAs [27] and are closely allied to those derived from the literature of formal calcium balance studies [28].

In 2011, the Institute of Medicine (IOM) published updated values for dietary reference intakes for calcium and vitamin D following a comprehensive review of the literature and scientific testimony. The full report is available online (see Tables 45.1 and 45.2). In general, the IOM recommendations are reasonably close to earlier elaborations. One emphasis of the IOM statement was that intakes above those shown as upper level increased the risk of adverse experiences, such as kidney stones.

Although a consensus conference on optimal calcium intake concluded that current habitual calcium intakes by both men and women are inadequate for optimal bone health [25], and several clinical trials demonstrated the ability of calcium supplementation to constrain the rate of bone loss and even reduce fractures [24,29–35], there remains in the medical and research communities some resistance to reaching consensus regarding the role of calcium inadequacy in the pathogenesis of osteoporosis. In part, this reflects considerable uncertainty in estimating the amount of calcium that people habitually consume. For example, dietary histories and food frequency questionnaires carry substantial imprecision. Also, it must be remembered that calcium is not absorbed in a vacuum but, rather, in the course of eating or drinking other foods, and its availability from foods is highly influenced by other nutrients. For example, although the calcium content of spinach is quite good, it is rendered essentially nonabsorbable by the presence of oxalate and perhaps other anions to which it binds [36]. Further consideration of the roles of phosphorus, sodium, and protein appears later.

For adults, calcium intakes in the range of 1000–1500 mg/day are recommended. Note that individuals in early to middle adult life, such as 20–50 years of age, have calcium intakes within reasonable proximity to recommended values and, by virtue of being young enough to undertake regular weight-bearing activity and to maintain normal reproductive function, generally have only modest stress on skeletal balance, as shown by very low rates of bone loss. After menopause (average age, 51 years), and certainly at more advanced ages, declines in endogenous reproductive hormones and in the insulin-like growth factor I axis, coupled with overall trends toward less physical activity, make it less likely that dietary

TABLE 45.1 Various Estimates of the Calcium Requirement (mg/day) in Women

Age (years)	1989 RDA[a]	NIH[b]	1997 AI[c]	Balance[d]
1–5	800	800	–	1100
6–10	800	800–1200	960	1100
11–24	1200	1200–1500	1560	1600
Pregnancy/lactation	1200	1200–1500	1200–1560	–
24–50/65	800	1000	1200	800–1000
65–	800	1500	1440	1500–1700

[a] *National Research Council [27].*
[b] *Recommendations for women as proposed by the Consensus Development Conference on Optimal Calcium Intake [25].*
[c] *The so-called adequate intakes of the new DRI values, multiplied by a factor of 1.2 to convert them into RDA format [26].*
[d] *Estimates derived from published balance studies [28].*
Source: Adapted from R.P. Heaney, Effects of protein on the calcium economy, in: B. Dawson-Hughes, R.P. Henry, Nutritional Aspects of Osteoporosis 2006, Elsevier, Amsterdam, 2007, 191–197 [48].

TABLE 45.2 Dietary Reference Intakes for Calcium (mg/day)

Age	Estimated Average Requirement	RDA	Upper Level Intake
Infants			
0–6 months			1000
6–12 months			1500
1–3 years	500	700	2500
4–8 years	800	1000	2500
9–18 years	1300	1300	3000
19–30 years	1000	1000	2500
31–50 years	1000	1000	2500
51–70 men	1000	1000	2000
51–70 women	1200	1200	2000
>70 years	1200	1200	2000
Pregnant/Lactating			
14–18 years	1100	1300	3000
19–50 years	800	1000	2500

Source: Institute of Medicine, The 2011 Report on dietary requirements for calcium and vitamin D, J. Clin. Endocrinol. Metab. 96 (2011) 53–58.

deficiency will be buffered and more likely that age-related bone loss will be aggravated.

c Phosphorus

Various domestic animals respond to excessive dietary phosphorus by increasing the endogenous concentration of PTH, resulting in negative calcium balance and bone loss. In such animals, optimal dietary ratios of calcium to phosphorus approach 1.0. This has led to a popular theory that because calcium:phosphorus ratios in human diets typically are well below 1.0, phosphorus overconsumption initiates a similar process in humans.

Firm evidence to support a role for phosphorus excess in human osteoporosis has not been forthcoming. In contrast, some evidence indicates that intestinal calcium absorption and balance are fairly impervious to very wide variations in daily phosphorus consumption [37,38]. Separate mention should be made concerning the role of phosphorus-containing soft drinks. Cola drinks contain phosphoric acid as their source of effervescence, and one reads frequently in the lay press that the high content of phosphorus in colas is an important contributor to developing osteoporosis. However, although excessive soft drink consumption may well contribute to poor bone status, this is so because these beverages have been substituted for milk, thus exchanging a calcium-rich drink for one that contains essentially no calcium [39].

d Protein

For many years, a concept has circulated as a subtext in osteoporosis research that consumption of excess protein, particularly from animal sources, is an important contributor to the development of osteoporosis. This is considered the consequence of the fact that protein catabolism generates ammonium ion from ammonia and sulfate from sulfur-containing amino acids. When protein intake increases, citrate and carbonate ions are released from bone to neutralize these acids, and, because urinary calcium is closely linked to renal acid excretion, urinary calcium rises. However, plant proteins contain amounts of sulfur similar to those in eggs, milk, and meats, and therefore increased intake of protein from either animal or plant sources similarly increases urinary calcium. Furthermore, the impact of protein on calcium balance depends to a major degree on other nutrients contained in the consumed food. For example, milk calcium compensates for urinary calcium losses generated by milk protein, potassium in such plants as legumes and grains decreases calcium excretion, and the high phosphorus content of meat offsets the calciuric effect of the protein [40].

Nutritional status assessments of patients with osteoporosis do not support a view that these patients have enjoyed a life of luxurious protein consumption. Barger-Lux et al. [41] reported that women with low calcium intakes generally consumed insufficient amounts of multiple nutrients, including protein. Sellmeyer et al. [42] reported higher protein intakes to be associated with greater age-related bone loss in the SOF cohort. However, a substantial body of evidence does not sustain this view. Protein consumption was reported to be an important positive predictor of bone mass in elderly women [43]. In the Framingham cohort, Hannan et al. [44] reported bone loss over time to be inversely related to protein intake, with rates of loss in the highest quartile of protein consumption less than one-third of those in the lowest quartile. Kerstetter et al. [45] conducted a series of controlled balance studies that showed that although calciuria increased with increased protein intake, this did not come from bone but was rather a reflection of improved intestinal calcium absorption. In a randomized controlled trial, Dawson-Hughes and Harris [46] showed that the improvement in BMD in subjects supplemented with calcium and vitamin D was confined mainly to individuals whose protein intake was in the highest tertile. Delmi et al. [47] found that supplemental protein improved recovery from hip fracture, accelerated hospital discharge, and also slowed bone loss in the contralateral hip. Heaney [48]

concluded that the weight of evidence shows high protein intake to be osteoprotective, but only if calcium intake is adequate, whereas the protective effect of calcium occurs only when protein intake is relatively high.

e Sodium

Urinary excretion of calcium and that of sodium are highly linked, and increased sodium intake is known to promote calcium excretion. A similar effect occurs with most diuretic agents (with the single exception of thiazides, which uncouple the handling of these two cations). Thus, it is not unreasonable to expect that increased dietary sodium might be conducive to negative calcium balance and aggravate bone loss. In a 2-year prospective trial, Devine et al. [49] examined the influence of urinary sodium excretion and dietary calcium intake on bone density of postmenopausal women. Urinary sodium excretion (a robust indication of intake) correlated negatively with changes in BMD, and the data suggested that halving sodium intake had the same effect on BMD as increasing daily calcium intake by 891 mg [49].

f Vitamin D

The importance of vitamin D to skeletal maintenance is thoroughly discussed elsewhere in this volume. When severe, vitamin D deficiency is associated with significant undermineralization of bone matrix, a condition known as osteomalacia [50]. At milder levels of vitamin D inadequacy, impaired calcium absorption promotes compensatory secretion of PTH, which increases bone remodeling and aggravates bone loss. Maintaining vitamin D adequacy is an important and widespread issue for the general population and for the vast majority of patients with osteoporosis. It appears that optimal vitamin D status is achieved at 25-hydroxyvitamin D concentrations of approximately 30 ng/mL (80 nmol/L). To achieve this goal, previously recommended vitamin D consumption of 400–800 units/day is not adequate, and doses of 1000–2000 units/day are preferable.

3 Reproductive Hormonal Status

Decades of studies in animals and humans support the concept that achieving and maintaining normal gonadal function is a critical determinant of bone health during pubertal bone acquisition and throughout adult life in both women and men. Indeed, the impact of menopausal loss of endogenous estrogen on skeletal balance can be so profound that many investigators have focused solely on the contribution of estrogen withdrawal to osteoporosis without regard for the other contributions described previously. We now understand that permanent loss of estrogen at menopause is not the only circumstance in which gonadal status has a skeletal impact. During earlier adult life, transient episodes of oligo- or amenorrhea may also be associated with at least transient bone loss [51].

The mechanisms by which estrogen withdrawal affects the skeleton involve multiple organs. The direct skeletal effect of the most potent circulating estrogen, 17-β estradiol, is to suppress the rate of bone remodeling. This is achieved by downregulating the formation of osteoclasts. In some rodents, this effect has been related to the suppression of an osteoclast-stimulating cytokine, interleukin-6 [52,53]. In other animal models and in humans, strong evidence has been presented for the participation of other cytokines, including interleukins and transforming growth factor-β [54]. In addition, estrogen withdrawal reduces osteoblast production of OPG, the decoy receptor for RANK-ligand discussed previously [55]. These cytokine effects are reversed when estrogen is replaced. The use of estrogen as pharmacologic therapy for the prevention and treatment of osteoporosis is described later.

IV DIAGNOSIS OF OSTEOPOROSIS

Because traditional radiographic techniques cannot distinguish osteoporosis until it is severe, diagnosis was, until recently, clinical, requiring a history of one or more low-trauma fractures. Although highly specific, such a grossly insensitive diagnostic criterion offered no assistance to physicians who hope to identify and treat affected individuals who have been fortunate not yet to have sustained a fracture. The focus in this section is the measurement of BMD. However, one must be cognizant of a wide variety of risk factors other than bone density that may profoundly influence an individual's risk for fracture. A partial list of these factors is presented in Table 45.3.

The introduction of accurate noninvasive bone mass measurements afforded the opportunity to estimate a person's fracture risk and to make an early diagnosis of osteoporosis. Large prospective studies have shown that a reduction in BMD of 1 standard deviation (SD) from the mean value for an age-specific population confers a two- or threefold increase in long-term fracture risk [56–59]. In a manner similar to that by which serum cholesterol concentration predicts risk for heart attack or blood pressure predicts risk for stroke, BMD measurements can successfully identify subjects at risk of fracture and can help physicians select those individuals who will derive greatest benefit for initiation of therapy. Indeed, the gradient of risk associated with a 1 SD difference in BMD is even greater than those for cholesterol and heart attack.

Several factors limit the ability of BMD measurements to predict an individual's fracture risk with great accuracy. The normative data against which BMD comparisons are most often made have been determined for Caucasian men and women and do not necessarily apply to other ethnic groups. This problem is gradually being overcome as ethnic-specific BMD data have begun to penetrate the literature. BMD is clearly related to body

TABLE 45.3 Partial List of Risk Factors for Osteoporosis and Related Fractures

Major Factors
• Personal history of fracture as an adult
• History of fragility fracture in a first-degree relative
• Low body weight (less than approximately 127 lb)
• Current smoking
• Use of oral corticosteroid therapy for more than 3 months
Additional Risk Factors
• Impaired vision
• Estrogen deficiency at an early age (<45 years)
• Thyroid hormone excess
• Intestinal malabsorption
• Some varieties of hypercalciuria
Selected Medications (cyclosporin and related agents, thiazolidinediones, possibly selective serotonin inhibitors)
• Dementia
• Poor health/frailty
• Recent falls
• Low calcium intake (lifelong)
• Immobilization or low physical activity
• Alcohol in amounts >2 drinks per day

Source: Adapted from National Osteoporosis Foundation, Physician's Guide to Prevention and Treatment of Osteoporosis, Excerpta Medica, Belle Mead, NJ, 1998 [1].

weight, but routine clinical bone mass assessments are not weight-adjusted. Various features of bone geometry that affect bone strength and fracture risk are not considered in the clinical interpretation of bone mass measurements. These include bone size, the distribution of bone mass around its bending axis (moments of inertia), and some derivative functions such as the hip axis length [59]. Moreover, bone mass measurements cannot distinguish individuals with low mass and intact microarchitecture from those with equal mass who have trabecular disruption and cortical porosity. They also cannot distinguish other aspects of bone quality.

In 1994, a group of senior investigators in this field offered a working definition of osteoporosis based exclusively on bone mass [60]. The reasoning behind this proposal, made on behalf of the World Health Organization, was that the clinical significance of osteoporosis lies exclusively in the occurrence of fracture, that bone mass predicts long-term fracture risk, and that selection of rigorous diagnostic criteria would minimize the number of patients who are incorrectly diagnosed. The authors suggested a cutoff BMD value of 2.5 SD below the average for healthy young adult women. Using this value, approximately 30% of postmenopausal women would be designated as osteoporotic, which gives a realistic projection of lifetime fracture rates. In addition, Kanis et al. [60] proposed that BMD values of 1 or 2 SD below the young adult mean be designated as "osteopenic." Such values identify individuals at modestly increased risk for fracture but for whom a diagnosis of osteoporosis would not be justified because it would mislabel far more individuals than would actually be expected ever to fracture.

This approach has proven useful for clinical management, but it has limitations. Its application to young people prior to their acquisition of peak bone mass would, of course, be inappropriate. The BMD measurement is subject to several confounding factors, including bone size and geometry. Because BMD correlations among skeletal sites are not strong, designating a person "normal" based on a single site, such as the lumbar spine, necessarily overlooks individuals with low bone density elsewhere, such as the hip. It seems reasonable to suppose that adjustment of bone density readings for such factors as body size, bone geometry, and ethnic background might improve the accuracy of this technique. Finally, studies indicate that although individuals with low BMD are at greater relative risk to fracture, many fractures in the population are experienced by individuals with normal bone mass [61–63]. Knowledge of a low bone density at a particular point in time offers no information regarding the adequacy of peak bone mass attained, the amount of bone that may have been lost, or the quality of bone that remains.

The World Health Organization completed an initiative to give individuals an estimate of their absolute 10-year fracture risk. It is based on the concept that BMD plus clinical risk factors predict fracture risk better than BMD or clinical risk factors alone. When BMD is not available, fracture risk can be calculated with only the clinical risk factors. This published initiative, called FRAX, is readily available for routine use via an online calculator (www.shef.ac.uk/FRAX). It is also included in current Dual-Energy X-ray Absorptiometry software and available as a smartphone application. Separate calculation tools are provided at this website for individuals living in many other countries worldwide. In many countries, osteoporosis treatment guides include intervention thresholds based on FRAX fracture risk calculations.

A Beyond BMD: The Question of Bone Quality

As stated in the introduction to this chapter, osteoporosis is a condition of decreased bone strength, where strength is a composite function of BMD and bone quality.

Several diverse qualitative characteristics have been described that directly influence bone strength. The more important factors—bone geometry, microarchitectural integrity, mineralization state, and remodeling rate—are briefly considered here. For more detailed information, the reader may consult Seeman and Delmas [64]. The first factor is overall bone geometry. A bone of greater diameter is better able than a smaller bone to withstand either a compressive or a bending force. One reason that men are relatively less susceptible to forearm fractures than are women is the fact that long bones of men are wider. A second feature, particularly in trabecular bone, is microarchitectural integrity. Normal trabecular bone is a honeycomb of highly connected vertical and horizontal trabeculae (Fig. 45.1A), and the holes, or spaces between the trabeculae, are fairly uniform. By contrast, osteoporotic trabecular bone gives the appearance of Swiss cheese (Fig. 45.1B). The holes are not uniform because excessive bone resorption has perforated the trabeculae, leaving confluent, sometimes extremely large holes that represent the permanent loss of entire trabecular units. A third important qualitative factor is the state of bone remodeling. It will be remembered that each remodeling event begins with the removal by osteoclasts of a divot of bone from trabecular surface. At any one time, then, there are hundreds of thousands of resorption holes, or *lacunae*, on bone surfaces. These holes are an inherent point of weakness to the bone, permitting very modest mechanical loads to overload the structure and fracture it. In mechanical engineering parlance, such points of weakness are called "stress concentrators." Mechanical stresses that are generated across an area of bone divert from their normal path of stress transmission to focus on the area of weakening, thereby overloading it. Thus, individuals with higher levels of bone remodeling activity will have more stress-concentrating lacunae on their bone surfaces, giving them a greater chance for fracture than somebody in a low remodeling state. Finally, the degree of bone mineralization is also an important feature of bone quality. Roughly speaking, except in extreme cases, the more mineralized the bone matrix, the greater its mechanical strength. Because a portion of bone continues to accumulate mineral as long as it exists, individuals with lower remodeling rates will have bone that is older, and therefore more mineralized and stronger, than individuals whose bone is remodeled more rapidly.

One message from this inquiry into bone quality is that a BMD measurement simply does not describe a sufficient amount of information about the nature of the skeleton ever to be a gold standard for the presence of osteoporosis. Unfortunately, currently, no suitable noninvasive measurements are available for routine assessment of bone quality in patients outside a research environment. Therefore, one is forced to rely on BMD measures to some degree. That being said, one also should not depend on changes in BMD over time as a true index of whether patients have improved or deteriorated in response to therapeutic interventions. One important indication of overall bone quality is whether a given person has already sustained a low-trauma fracture. Evidence has been published that individuals with such fractures have more serious disruption of qualitative measures seen on bone biopsies than individuals who have not fractured [65]. Therefore, the presence of a vertebral compression fracture on a radiograph of the spine may reasonably permit the physician to conclude that the patient has poor bone quality.

V OSTEOPOROSIS PREVENTION AND TREATMENT

A Hygienic Management

The term *hygienic* is used here in the sense of being nonpharmacologic. It refers to the appropriate attention to lifestyle factors that either protect or damage bone. These principles have universal application regardless of whether one wishes to forestall bone loss and development of osteoporosis or treat established disease.

1 Physical Activity

As described previously, the skeleton adapts to its mechanical environment so that the steady-state amount of bone reflects daily exposure to mechanical forces. Although the skeletal response to vigorous exercise, such as weight lifting (resistance activity) or running (endurance activity), may be greater than that observed with walking, the majority of middle-aged and older individuals are more likely to persist long term with a program of walking. A progressive schedule of walking 30 minutes or more several days each week provides a mechanical load to the legs and axial skeleton and should form the basis of skeletal maintenance for ambulatory persons in their middle and advanced years. Younger individuals should certainly be encouraged to pursue more vigorous activities, but it must be remembered that the effects of high-load environments persist only so long as the high loading schedule continues. If a person stops training, the bone perceives a reduction in mechanical environment and adaptive responses will lead to loss of bone until a new steady state is reached.

2 Dietary Factors

With respect to overall dietary intakes, substantial weight loss during adult life is a risk factor for fractures. Weight-loss programs are associated with measurable decreases in BMD, and the impact of so-called yo-yo weight

fluctuations, although unclear, carries some skeletal risk. As discussed previously, the nutrients of most relevance to skeletal maintenance are calcium and vitamin D. A daily calcium intake of 1200−1500 mg should be the goal for most healthy adults. Consuming a quart of nonfat milk each day could approach this, but it is very unlikely that most adults, even dairy enthusiasts, will consistently accomplish that. Each quart of milk contains approximately 1100 mg calcium, although this may vary somewhat depending on its fat content. Because calcium is contained within the aqueous phase of milk, an equal volume of low-fat or skim milk has more water, and hence more calcium, than whole milk. In addition, some brands of reduced-fat milk are enriched by the addition of milk solids, which further increases calcium content.

Many segments of the population experience loss of lactase, the enzyme necessary for the hydrolysis of the major milk sugar lactose. Many afflicted individuals experience bloating, cramps, or other symptoms of intestinal distress if they consume lactose-containing dairy products. Several strategies can be recommended for such individuals. Lactose-free milk is readily available in markets. Lactose in this product has been prehydrolyzed to its constituent sugars, glucose and galactose, by incubation with commercial lactase. This milk has a slightly sweet taste due to the taste of its hexose sugars. For those who do not care for this taste, lactase tablets can be taken immediately prior to drinking milk. Yogurt and other products in which lactose is hydrolyzed provide an excellent substitute for milk. Many cheeses are rich in calcium (not cottage cheese, however) but have the complicating feature of high sodium content. The calcium:sodium ratio of most liquid dairy products is approximately 1.0, but the ratio is well below 1 for hard cheeses. Given the relationship between sodium intake and calciuria [49], depending on cheese calcium consumption may not be effective.

Other reasonably good food sources of calcium include small fish (anchovies, herrings, and sardines), nuts, and some green vegetables (broccoli). However, it is quite difficult to attain calcium adequacy with these foods alone without also consuming dairy products. For example, it may require 6 cups of broccoli to provide as much calcium as would be consumed in a single 8-ounce glass of milk.

For many individuals, a calcium supplement offers the most convenient approach to reaching target levels of calcium intake. Many calcium salts are available on an over-the-counter basis. Calcium carbonate has the advantage of having the highest percentage by weight in calcium (40% of calcium carbonate is calcium) so that the fewest number of pills need to be taken. Calcium citrate is a reliable calcium supplement. It has been alleged that individuals who have achlorhydria or are taking medications that reduce gastric acid secretion better absorb the citrate salt than the carbonate. However, it has been shown that when calcium carbonate is taken with food, there is sufficient acidity in the food to permit normal calcium absorption [66]. Therefore, my general recommendation for individuals who will likely not consume more than 1000 mg calcium per day through food alone is to take a 500 mg calcium supplement as calcium carbonate each morning with breakfast. For individuals with osteoporosis who are receiving pharmacological treatment, I increase that recommendation to 1000 mg calcium/day.

In 2008, Bolland et al. [67] reported on a calcium intervention trial in older women, in which consumption of calcium as supplement, but not as food, was associated with an increased risk for cardiovascular complications, including myocardial infarction. Subsequently, the same group published a meta-analysis from which they concluded that "calcium supplements without vitamin D are associated with an increased risk of myocardial infarction" [68]. This publication created a storm of controversy. Although the meta-analysis was constructed from multiple clinical trials, the most cases came from the single trial reported by Bolland and colleagues [67]. Myocardial infarction was not an end point of that trial, and cases were not adjudicated. The 2-year pill compliance rate was only 55% and was lower yet in the group receiving calcium. Although the conclusions were based on an intervention in older women, a number of the studies included in the meta-analysis included both men and women, and none independently showed even a trend toward a cardiovascular adverse effect. All these factors could introduce bias into group comparisons. Moreover, some independent studies failing to support the views of Bolland et al. were not represented in the meta-analysis [68]. In particular, a well-designed and executed clinical trial in which cardiovascular end points were prespecified showed no impact of calcium supplementation on either atherosclerotic vascular mortality or first hospitalization, either during the 5-year clinical trial or during a 4.5-year follow-up period [69]. Given the importance of this topic, a committee from the American Society for Bone and Mineral Research evaluated the available data and concluded that "the weight of evidence is insufficient to conclude that calcium supplements cause adverse cardiovascular events; however, the debate continues."

B Pharmacologic Therapy

The variety of approved drugs for the prevention and treatment of osteoporosis has expanded enormously during approximately the past decade (Table 45.4). These are grouped into two general categories—antiresorptive (sometimes called anticatabolic) and bone-forming (or anabolic). The first category contains the great majority of registered products. Although the fundamentals of their

TABLE 45.4 Approved Medications for the Prevention and Treatment of Osteoporosis

Antiresorptive
• Calcium/vitamin D
• Estrogens
• Bisphosphonates
• Alendronate
• Risedronate
• Ibandronate
• Zoledronatic acid
• Calcitonin
• Strontium ranelate (not approved in United States)
Bone-forming
• Teriparatide
• PTH (1–84) (not approved in United States)

mechanism of action differ from one product to the next, they all act ultimately to inhibit either the development of osteoclasts from their precursors or the activity of mature osteoclasts. Such actions confer skeletal protection in two ways, both of which are predictable consequences of slowing the remodeling rate by 30–70%, as per the previous discussion of bone quality. First, and perhaps most important, at least during the first year or so of therapy, reducing the creation of new resorption lacunae results in a major reduction in the prevalence of stress concentrators on bone surfaces. Second, because any given point on the bone surface will survive longer when the remodeling rate is low, the bone continues to gain mineral for a longer period of time and ultimately becomes relatively hypermineralized.

By contrast, only one bone-forming agent—the 1–34 fragment of human PTH (teriparatide; see later discussion)—is approved, as of this writing, in the United States for the treatment of osteoporosis. Its actions involve the direct stimulation of bone-forming osteoblasts.

Selection of appropriate therapies for individual patients is beyond the scope of this chapter. The following material briefly describes each of these approved products.

1 Antiresorptive Agents

a Calcium and Vitamin D

Strictly speaking, calcium and vitamin D have an antiresorptive activity on bone. By slightly elevating blood calcium concentrations, endogenous PTH secretion is reduced and overall bone remodeling decreases. The role of calcium and vitamin D in skeletal maintenance was described previously. Calcium administration to osteoporotic women with previous vertebral fractures has been shown to decrease the risk for subsequent fracture [33] and, when combined with a modest amount of vitamin D, has been shown to reduce the incidence of hip fracture in elderly women [24]. Suffice it to say that calcium and vitamin D adequacy are essential components of all successful therapies, and the general approach described previously must be pursued if any pharmacologic regimen is to succeed. Indeed, therapeutic failures are frequently attributable to inadequate vitamin D status. Note that all major osteoporosis trials were designed to show an effect of drug on bone turnover, BMD, and fracture incidence superior to that of the control regimens, which invariably consisted of calcium and vitamin D.

b Estrogen

The use of estrogen to treat postmenopausal osteoporosis has been popular for at least 50 years. The era of the 1970s and 1980s saw the publication of many small clinical trials demonstrating the effects of various estrogen and estrogen/progestin combinations in early and postmenopausal women to improve calcium balance, to suppress markers of bone turnover, to increase BMD, and in small series to protect against vertebral compression fractures [70]. Because the available studies did not demonstrate a compelling case that estrogen administration to women with established osteoporosis led to a significant reduction in fracture incidence, U.S. Food and Drug Administration (FDA) approval of estrogen was specified for prevention of osteoporosis, not for treatment. With publication of results from the Women's Health Initiative, we now have conclusive evidence for the nonvertebral fracture efficacy of estrogen, even in women who were not osteoporotic or at high risk for fracture [71]. Unfortunately, the Women's Health Initiative also established that estrogen, particularly when given in combination with progestin, increased the development of breast cancer, myocardial infarction, and other cardiovascular events and potentially contributed to cognitive decline. Thus, although estrogens remain highly effective when treating women with hot flashes, current opinion holds that they should be used at the lowest possible dose and for the shortest possible duration and are not recommended as long-term therapy for prevention or treatment of osteoporosis.

c Selective Estradiol Receptor Modulators

These compounds are neither hormones nor estrogens but, rather, molecules that interact with the estradiol receptor in multiple tissues of the body. For a comprehensive description of selective estradiol receptor modulator

(SERM) physiology, the reader is referred to the review of Siris and Muchmore [72]. Briefly summarized, unlike estrogens, SERMs interact with the estradiol receptor and activate estrogen-regulated genes in a manner that is tissue specific. For example, like estrogen, tamoxifen stimulates uterine hyperplasia and suppresses bone remodeling, but unlike estrogen, it inhibits estrogen actions at the breast. On the other hand, raloxifene has no effect on the endometrium, acts like estrogen on bone, and antagonizes estrogen action at the breast. Although several such molecules were introduced into clinical medicine before their mechanisms of action were clarified (e.g., tamoxifen), recent years have seen the development of numerous molecules in this class for the intended purpose of treating osteoporosis. As of this writing, only two SERMs, raloxifene (Evista, Eli Lilly & Co.) and bazedoxifine, in combination with a low dose of conjugated estrogens (Duavee, Pfizer Inc.) are FDA approved for preventing osteoporosis. Raloxifene has been shown to prevent the development of frank osteoporosis in women with low bone mass and also to offer substantial long-term protection against vertebral fracture in women with established osteoporosis [73], thus it is approved also for treatment of established osteoporosis. At the approved dose of 60 mg daily, raloxifene offers significant protection against the development of estrogen receptor-positive breast cancer, for which it also has received FDA approval.

d Bisphosphonates

Bisphosphonates are analogs of the naturally occurring phosphate ester pyrophosphate, in which a carbon atom has replaced the central oxygen bridge. This substitution renders the compounds nonsusceptible to hydrolysis by alkaline phosphatase, a ubiquitous enzyme that hydrolyzes pyrophosphate. Bisphosphonates are poorly absorbed from the gut, but once absorbed they are taken up by bone. The presence of two phosphate groups permits the molecule to bind avidly to hydroxyapatite so that the half-life of bisphosphonates in the skeleton may be a matter of many years. In theory, osteoclasts imbibe the bisphosphonate during the course of bone resorption, resulting in osteoclast death and a decrease in resorption. Differences in one of the two side chains underlie differences in action among agents of this class. The first generation of bisphosphonates acted to inhibit intermediary metabolism. More recent compounds that have amino groups on the side chain act in a manner similar to the statin class of antilipid drugs—that is, inhibiting the mevalonate synthesis pathway, but at a level (farnesyl diphosphate synthetase) that is further downstream, leading to interference with prenylation of plasma membrane lipids [74]. First developed and introduced for the treatment of Paget's disease of bone in the 1960s, several bisphosphonates have shown significant improvement in BMD and reductions in fracture. Three oral bisphosphonates are currently approved for prevention and treatment of osteoporosis: alendronate (Fosamax) [75], risedronate (Actonel) [76,77], and ibandronate (Boniva) [78]. The last may also be administered by intravenous infusion. Another very potent bisphosphonate, zoledronic acid, has received FDA approval for treatment of osteoporosis. This agent is administered only once each year by intravenous infusion.

In recent years, it has become apparent that some patients receiving long-term potent bisphosphonates may experience potentially serious complications of treatment, including osteonecrosis of the jaw (ONJ) and nonclassical or "atypical" hip fractures [79]. Although the precise mechanisms by which such complications may be induced remain unsettled, it appears likely that they reflect in some manner the degree to which bone remodeling has been suppressed. For example, because of its role in chewing, the mandible receives some of the heaviest mechanical loads of any bone in the body. To maintain mandibular health and prevent fatigue damage, it is necessary to have a robust and responsive remodeling system. In the presence of potent antiresorptive therapy, remodeling might not be adequate to prevent tissue breakdown and subsequent necrosis. Similarly, a number of case reports and patient series have described the occurrence of fractures in the subtrochanteric region of the femur in patients exposed to long-term bisphosphonates. These transverse fractures show a characteristic radiographic appearance, with thickening of the bony cortex and "beaking" of the fracture fragment. In a very large population-based study, it was determined that although there is a small added risk for such atypical fractures, the overall benefit:risk ratio for bisphosphonates remained substantially positive in that the overall fracture incidence in bisphosphonate-treated patients was considerably lower than that of nontreated patients [80]. Nonetheless, a trend in treatment strategy has attended the emergence of ONJ and atypical fractures, in that many experts now recommend bisphosphonate therapy be stopped following 5 consecutive years of treatment, with subsequent monitoring of BMD so that treatment can be restarted if and when bone loss recurs. The degree to which undertaking such a "drug holiday" may promote further bone loss and fractures remains under study.

e Calcitonin

This 32-amino acid peptide is a natural hormone throughout the vertebrate phylum. Its primary physiological role appears to be to reduce the rate of bone remodeling by inhibiting the action of mature osteoclasts. Calcitonin obtained from salmon is considerably more potent than

the human hormone and has been approved for treatment of osteoporosis [81]. Although calcitonin can be given by subcutaneous injection, most patients take the drug as a nasal spray (Miacalcin). In approved doses, calcitonin is a relatively weak antiresorptive drug with efficacy characteristics that are far less pronounced than those of the other approved antiresorptive agents.

f Denosumab

In Section II.D, Intercellular Communication Among Bone Cells: Triggers and Constraints on Remodeling, I described the role of the RANK−RANK-ligand (RANKL) system for regulating bone turnover. In 2010, the FDA approved denosumab, a human monoclonal antibody that specifically binds to RANKL, prevents the activation of RANK on the surface of osteoclasts and their precursors, and thereby inhibits osteoclast formation and function, substantially decreasing the rate of bone remodeling [82]. In its pivotal clinical trial, denosumab reduced the occurrence of all categories of fragility fracture: vertebral, hip, and nonvertebral. Based on its mechanism of action, denosumab is considered a potent antiresorptive drug. Marketed under the trade name Prolia (Amgen), it is approved for treatment of men and postmenopausal women with osteoporosis at high risk of fracture. The drug is administered by vein every 6 months and is generally well tolerated. Some concern has been expressed regarding the tendency for patients to experience lower blood calcium concentrations when treated with denosumab, and hypocalcemia is considered a contraindication for its use. In addition, in clinical trials, serious infections leading to hospitalization were more frequent in the denosumab group than with placebo. These include bacterial endocarditis, skin infections, and infections in the abdomen and urinary tract. ONJ has been reported in a few patients receiving denosumab.

g Strontium Ranelate

Strontium has received approval in Europe and Asia for treatment of osteoporosis. It becomes incorporated directly into the bone mineral, which creates an artifactual increase in BMD. However, strontium does appear to be an effective antiresorptive compound and has been shown to reduce fracture risk. Because no studies of this compound have been conducted in the United States, it does not appear that strontium can ever receive FDA approval and so it is unlikely to be introduced into the U.S. market.

2 Bone Formation Therapy

a Teriparatide (Forteo and Forsteo in Europe)

Teriparatide is the generic term for the 1−34 fragment of human PTH. It is approved as a single daily subcutaneous injection for up to 2 years at a dose of 20 μg/day. Teriparatide is the first bone anabolic agent approved for treatment of osteoporosis. (The full-length PTH (1−84) molecule has not received approval in the United States but is marketed in Europe. Its actions are qualitatively similar to those of teriparatide.) Teriparatide directly stimulates osteoblasts to form new bone and results in considerably greater increases in BMD than are observed with antiresorptive drugs. In addition, teriparatide uniquely repairs the disrupted microarchitecture of trabecular bone to a normal pattern and also increases the thickness of cortical bone. These effects result in substantial reduction in both vertebral and nonvertebral fracture [83]. Teriparatide given very long term at high dose to Fischer 344 rats led to a high rate of osteosarcoma. Other animal models have shown no such effect, and although a relationship to human carcinogenesis seems very unlikely, one cannot be certain that no relationship exists. Because duration of therapy was a critical element for carcinogenesis in rats, teriparatide use is currently restricted to 2 years. Teriparatide is marketed by Eli Lilly & Co. as Forteo (and as Forsteo in Europe) for the treatment of men and postmenopausal women with osteoporosis whose physicians consider them at high risk for fracture. As of this writing, teriparatide has been generally available for more than 13 years, and no carcinogenesis signal has yet appeared. As opposed to several of the antiresorptive agents, it is not a drug for prevention of osteoporosis.

VI CONCLUSION

Efforts are underway to bring forth additional compounds of both the antiresorptive and bone-formation classes. Other developmental targets currently focus on bone-forming agents, and new PTH analogs and modes of delivery for teriparatide are in human trials, as is suppression of a bone formation-inhibiting molecule, sclerostin. Still largely in animal studies, strategies to activate a newly discovered anabolic pathway in bone, the Wnt pathway, have begun.

Thus, the future of pharmacologic therapy for osteoporosis seems reasonably bright. However, the overall outlook for this disease remains clouded. With aging of the population, trends toward increasingly sedentary life, and substandard intakes of critical nutrients, the worldwide burden of osteoporotic fractures is likely to increase dramatically. Even now, it is difficult in the United States to get appropriate diagnosis and treatment for afflicted patients, even those with multiple fractures. With growing competition from other aspects of health care and with continued threats of onerous governmental and health insurance constraints on reimbursement for osteoporosis diagnosis and treatment, it is not at all certain that an explosive increase in fragility fractures and their consequences will be avoided.

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Appendix

Dietary Reference Intakes (DRIs): Estimated Average Requirements (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Calcium (mg/day)	CHO (g/day)	Protein (g/kg/day)	Vitamin A (μg/day)[a]	Vitamin C (mg/day)	Vitamin D (μg/day)	Vitamin E (mg/day)[b]	Thiamin (mg/day)	Riboflavin (mg/day)	Niacin (mg/day)[c]	Vitamin B_6 (mg/day)	Folate (μg/day)[d]	Vitamin B_{12} (μg/day)	Copper (μg/day)	Iodine (μg/day)	Iron (mg/day)	Magnesium (mg/day)	Molybdenum (μg/day)	Phosphorus (mg/day)	Selenium (μg/day)	Zinc (mg/day)
Infants																					
0–6 months																					
6–12 months			1.0													6.9					2.5
Children																					
1–3 years	500	100	0.87	210	13	10	5	0.4	0.4	5	0.4	120	0.7	260	65	3.0	65	13	380	17	2.5
4–8 years	800	100	0.76	275	22	10	6	0.5	0.5	6	0.5	160	1.0	340	65	4.1	110	17	405	23	4.0
Males																					
9–13 years	1100	100	0.76	445	39	10	9	0.7	0.8	9	0.8	250	1.5	540	73	5.9	200	26	1055	35	7.0
14–18 years	1100	100	0.73	630	63	10	12	1.0	1.1	12	1.1	330	2.0	685	95	7.7	340	33	1055	45	8.5
19–30 years	800	100	0.66	625	75	10	12	1.0	1.1	12	1.1	320	2.0	700	95	6	330	34	580	45	9.4
31–50 years	800	100	0.66	625	75	10	12	1.0	1.1	12	1.1	320	2.0	700	95	6	350	34	580	45	9.4
51–70 years	800	100	0.66	625	75	10	12	1.0	1.1	12	1.4	320	2.0	700	95	6	350	34	580	45	9.4
>70 years	1000	100	0.66	625	75	10	12	1.0	1.1	12	1.4	320	2.0	700	95	6	350	34	580	45	9.4
Females																					
9–13 years	1100	100	0.76	420	39	10	9	0.7	0.8	9	0.8	250	1.5	540	73	5.7	200	26	1055	35	7.0
14–18 years	1100	100	0.71	485	56	10	12	0.9	0.9	11	1.0	330	2.0	685	95	7.9	300	33	1055	45	7.3
19–30 years	800	100	0.66	500	60	10	12	0.9	0.9	11	1.1	320	2.0	700	95	8.1	255	34	580	45	6.8
31–50 years	800	100	0.66	500	60	10	12	0.9	0.9	11	1.1	320	2.0	700	95	8.1	265	34	580	45	6.8
51–70 years	1000	100	0.66	500	60	10	12	0.9	0.9	11	1.3	320	2.0	700	95	5	265	34	580	45	6.8
>70 years	1000	100	0.66	500	60	10	12	0.9	0.9	11	1.3	320	2.0	700	95	5	265	34	580	45	6.8
Pregnancy																					
14–18 years	1000	135	0.88	530	66	10	12	1.2	1.2	14	1.6	520	2.2	785	160	23	335	40	1055	49	10.5
19–30 years	800	135	0.88	550	70	10	12	1.2	1.2	14	1.6	520	2.2	800	160	22	290	40	580	49	9.5
31–50 years	800	135	0.88	550	70	10	12	1.2	1.2	14	1.6	520	2.2	800	160	22	300	40	580	49	9.5
Lactation																					
14–18 years	1000	160	1.05	885	96	10	16	1.2	1.3	13	1.7	450	2.4	985	209	7	300	35	1055	59	10.9
19–30 years	800	160	1.05	900	100	10	16	1.2	1.3	13	1.7	450	2.4	1000	209	6.5	255	36	580	59	10.4
31–50 years	800	160	1.05	900	100	10	16	1.2	1.3	13	1.7	450	2.4	1000	209	6.5	265	36	580	59	10.4

[a] *As retinol activity equivalents (RAEs): 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE.*

[b] *As α-tocopherol: α-Tocopherol includes RRR-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the* 2R-*stereoisomeric forms of α-tocopherol (*RRR-, RSR-, RRS-, *and* RSS-α-tocopherol*) that occur in fortified foods and supplements. It does not include the* 2S-*stereoisomeric forms of α-tocopherol (*SRR-, SSR-, SRS-, *and* SSS-α-tocopherol*), also found in fortified foods and supplements.*

[c] *As niacin equivalents (NE): 1 mg of niacin = 60 mg of tryptophan.*

[d] *As dietary folate equivalents (DFE): 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach.*

Note: An Estimated Average Requirement (EAR) is the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group. EARs have not been established for vitamin K, pantothenic acid, biotin, choline, chromium, fluoride, manganese, or other nutrients not yet evaluated via the DRI process.

Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002–2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Vitamins (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Vitamin A (μg/day)[a]	Vitamin C (mg/day)	Vitamin D (μg/day)[b,c]	Vitamin E (mg/day)[d]	Vitamin K (μg/day)	Thiamin (mg/day)	Riboflavin (mg/day)	Niacin (mg/day)[e]	Vitamin B_6 (mg/day)	Folate (μg/day)[f]	Vitamin B_{12} (μg/day)	Pantothenic Acid (mg/day)	Biotin (μg/day)	Choline (mg/day)[g]
Infants														
0–6 months	400*	40*	10	4*	2.0*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
6–12 months	500*	50*	10	5*	2.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
Children														
1–3 years	**300**	**15**	**15**	**6**	30*	**0.5**	**0.5**	**6**	**0.5**	**150**	**0.9**	2*	8*	200*
4–8 years	**400**	**25**	**15**	**7**	55*	**0.6**	**0.6**	**8**	**0.6**	**200**	**1.2**	3*	12*	250*
Males														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**900**	**75**	**15**	**15**	75*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	25*	550*
19–30 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
31–50 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
51–70 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
>70 years	**900**	**90**	**20**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
Females														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**700**	**65**	**15**	**15**	75*	**1.0**	**1.0**	**14**	**1.2**	**400**[i]	**2.4**	5*	25*	400*
19–30 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
31–50 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
51–70 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
>70 years	**700**	**75**	**20**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
Pregnancy														
14–18 years	**750**	**80**	**15**	**15**	75*	**1.4**	**1.4**	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
19–30 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
31–50 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
Lactation														
14–18 years	**1200**	**115**	**15**	**19**	75*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
19–30 years	**1300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
31–50 years	**1300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*

[a]As retinol activity equivalents (RAEs): 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE.

[b]As cholecalciferol: 1 μg cholecalciferol = 40 IU vitamin D.

[c]Under the assumption of minimal sunlight.

[d]As α-tocopherol: α-Tocopherol includes RRR-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the 2R-stereoisomeric forms of α-tocopherol (RRR-, RSR-, RRS-, and RSS-α-tocopherol) that occur in fortified foods and supplements. It does not include the 2S-stereoisomeric forms of α-tocopherol (SRR-, SSR-, SRS-, and SSS-α-tocopherol), also found in fortified foods and supplements.

[e]As niacin equivalents (NE): 1 mg of niacin = 60 mg of tryptophan; 0–6 months = preformed niacin (not NE).

[f]As dietary folate equivalents (DFE): 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach.

[g]Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

[h]Because 10–30% of older people may malabsorb food-bound B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with B_{12} or a supplement containing B_{12}.

[i]In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 μg from supplements or fortified foods in addition to intake of food folate from a varied diet.

[j]It is assumed that women will continue consuming 400 μg from supplements or fortified food until their pregnancy is confirmed and they enter prenatal care, which ordinarily occurs after the end of the periconceptional period—the critical time for formation of the neural tube.

Note: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents from being able to specify with confidence the percentage of individuals covered by this intake.

Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Calcium (mg/day)	Chromium (µg/day)	Copper (µg/day)	Fluoride (mg/day)	Iodine (µg/day)	Iron (mg/day)	Magnesium (mg/day)	Manganese (mg/day)	Molybdenum (µg/day)	Phosphorus (mg/day)	Selenium (µg/day)	Zinc (mg/day)	Potassium (g/day)	Sodium (g/day)	Chloride (g/day)
Infants															
0–6 months	200*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	0.4*	0.12*	0.18*
6–12 months	260*	5.5*	220*	0.5*	130*	**11**	75*	0.6*	3*	275*	20*	**3**	0.7*	0.37*	0.57*
Children															
1–3 years	**700**	11*	**340**	0.7*	**90**	**7**	**80**	1.2*	**17**	**460**	**20**	**3**	3.0*	1.0*	1.5*
4–8 years	**1000**	15*	**440**	1*	**90**	**10**	**130**	1.5*	**22**	**500**	**30**	**5**	3.8*	1.2*	1.9*
Males															
9–13 years	**1300**	25*	**700**	2*	**120**	**8**	**240**	1.9*	**34**	**1250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1300**	35*	**890**	3*	**150**	**11**	**410**	2.2*	**43**	**1250**	**55**	**11**	4.7*	1.5*	2.3*
19–30 years	**1000**	35*	**900**	4*	**150**	**8**	**400**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
31–50 years	**1000**	35*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
51–70 years	**1000**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.3*	2.0*
>70 years	**1200**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.2*	1.8*
Females															
9–13 years	**1300**	21*	**700**	2*	**120**	**8**	**240**	1.6*	**34**	**1250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1300**	24*	**890**	3*	**150**	**15**	**360**	1.6*	**43**	**1250**	**55**	**9**	4.7*	1.5*	2.3*
19–30 years	**1000**	25*	**900**	3*	**150**	**18**	**310**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
31–50 years	**1000**	25*	**900**	3*	**150**	**18**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
51–70 years	**1200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.3*	2.0*
>70 years	**1200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.2*	1.8*
Pregnancy															
14–18 years	**1300**	29*	**1000**	3*	**220**	**27**	**400**	2.0*	**50**	**1250**	**60**	**12**	4.7*	1.5*	2.3*
19–30 years	**1000**	30*	**1000**	3*	**220**	**27**	**350**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
31–50 years	**1000**	30*	**1000**	3*	**220**	**27**	**360**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
Lactation															
14–18 years	**1300**	44*	**1300**	3*	**290**	**10**	**360**	2.6*	**50**	**1250**	**70**	**13**	5.1*	1.5*	2.3*
19–30 years	**1000**	45*	**1300**	3*	**290**	**9**	**310**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*
31–50 years	**1000**	45*	**1300**	3*	**290**	**9**	**320**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*

Note: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents from being able to specify with confidence the percentage of individuals covered by this intake.
Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); and Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Total Water and Macronutrients (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Total Water[a] (L/day)	Carbohydrate (g/day)	Total Fiber (g/day)	Fat (g/day)	Linoleic Acid (g/day)	α-Linolenic Acid (g/day)	Protein[b] (g/day)
Infants							
0–6 months	0.7*	60*	ND	31*	4.4*	0.5*	9.1*
6–12 months	0.8*	95*	ND	30*	4.6*	0.5*	**11.0**
Children							
1–3 years	1.3*	**130**	19*	ND[c]	7*	0.7*	**13**
4–8 years	1.7*	**130**	25*	ND	10*	0.9*	**19**
Males							
9–13 years	2.4*	**130**	31*	ND	12*	1.2*	**34**
14–18 years	3.3*	**130**	38*	ND	16*	1.6*	**52**
19–30 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
31–50 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
51–70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
>70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
Females							
9–13 years	2.1*	**130**	26*	ND	10*	1.0*	**34**
14–18 years	2.3*	**130**	26*	ND	11*	1.1*	**46**
19–30 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
31–50 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
51–70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
>70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
Pregnancy							
14–18 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
19–30 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
31–50 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
Lactation							
14–18	3.8*	**210**	29*	ND	13*	1.3*	**71**
19–30 years	3.8*	**210**	29*	ND	13*	1.3*	**71**
31–50 years	3.8*	**210**	29*	ND	13*	1.3*	**71**

[a]Total *water includes all water contained in food, beverages, and drinking water.*
[b]*Based on gram protein per kg of body weight for the reference body weight, for example, for adults 0.8 g/kg body weight for the reference body weight.*
[c]*ND, not determined.*
Note: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDA) in **bold type** and Adequate Intakes (AI) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents from being able to specify with confidence the percentage of individuals covered by this intake.
Sources: From Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002–2005) and Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005). The report may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Acceptable Macronutrient Distribution Ranges (Food and Nutrition Board, Institute of Medicine, National Academies)

Macronutrient	Range (Percent of Energy)		
	Children, 1–3 years	Children, 4–18 years	Adults
Fat	30–40	25–35	20–35
n-6 Polyunsaturated fatty acids[a] (linoleic acid)	5–10	5–10	5–10
n-3 Polyunsaturated fatty acids[a] (α-linolenic acid)	0.6–1.2	0.6–1.2	0.6–1.2
Carbohydrate	45–65	45–65	45–65
Protein	5–20	10–30	10–35

[a]*Approximately 10% of the total can come from longer-chain* n-*3 or* n-*6 fatty acids.*
Source: From Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002–2005). The report may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Acceptable Macronutrient Distribution Ranges (Food and Nutrition Board, Institute of Medicine, National Academies)

Macronutrient	Recommendation
Dietary cholesterol	As low as possible while consuming a nutritionally adequate diet
Trans fatty acids	As low as possible while consuming a nutritionally adequate diet
Saturated fatty acids	As low as possible while consuming a nutritionally adequate diet
Added sugars[a]	Limit to no more than 25% of total energy

[a]*Not a recommended intake. A daily intake of added sugars that individuals should aim for to achieve a healthful diet was not set.*
Source: From Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002–2005). The report may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Vitamins (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Vitamin A (μg/day)[a]	Vitamin C (mg/day)	Vitamin D (μg/day)	Vitamin E (mg/day)[b,c]	Vitamin K	Thiamin	Riboflavin	Niacin (mg/day)[c]	Vitamin B_6 (mg/day)	Folate (μg/day)[c]	Vitamin	Pantothenic Acid	Biotin	Choline (g/day)	Carotenoids[d]
Infants															
0–6 months	600	ND[e]	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6–12 months	600	ND	38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Children															
1 – 3 years	600	400	63	200	ND	ND	ND	10	30	300	ND	ND	ND	1.0	ND
4 – 8 years	900	650	75	300	ND	ND	ND	15	40	400	ND	ND	ND	1.0	ND
Males															
9 – 13 years	1700	1200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14 – 18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31 – 50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
51 – 70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
>70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Females															
9 – 13 years	1700	1200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14 – 18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19 – 30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31 – 50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
51 – 70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
>70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Pregnancy															
14 – 18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19 – 30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31 – 50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Lactation															
14 – 18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19 – 30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31 – 50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND

[a] *As preformed vitamin A only.*
[b] *As α-tocopherol; applies to any form of supplemental α-tocopherol.*
[c] *The ULs for vitamin E, niacin, and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.*
[d] *β-Carotene supplements are advised only to serve as a provitamin A source for individuals at risk of vitamin A deficiency.*
[e] *ND, not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.*

Note: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Arsenic[a]	Boron (mg/day)	Calcium (mg/day)	Chromium	Copper (µg/day)	Fluoride (mg/day)	Iodine (µg/day)	Iron (mg/day)	Magnesium (mg/day)[b]	Manganese (mg/day)	Molybdenum (µg/day)	Nickel (mg/day)	Phosphorus (g/day)	Selenium (µg/day)	Silicon[c]	Vanadium (mg/day)[d]	Zinc (mg/day)	Sodium (g/day)	Chloride (g/day)
Infants																			
0–6 months	ND[e]	ND	1000	ND	ND	0.7	ND	40	ND	ND	ND	ND	ND	45	ND	ND	4	ND	ND
6–12 months	ND	ND	1500	ND	ND	0.9	ND	40	ND	ND	ND	ND	ND	60	ND	ND	5	ND	ND
Children																			
1–3 years	ND	3	2500	ND	1000	1.3	200	40	65	2	300	0.2	3	90	ND	ND	7	1.5	2.3
4–8 years	ND	6	2500	ND	3000	2.2	300	40	110	3	600	0.3	3	150	ND	ND	12	1.9	2.9
Males																			
9–13 years	ND	11	3000	ND	5000	10	600	40	350	6	1100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	3	400	ND	1.8	40	2.3	3.6
Females																			
9–13 years	ND	11	3000	ND	5000	10	600	40	350	6	1100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	3	400	ND	1.8	40	2.3	3.6
Pregnancy																			
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	3.5	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	3.5	400	ND	ND	40	2.3	3.6
61–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	3.5	400	ND	ND	40	2.3	3.6
Lactation																			
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	ND	40	2.3	3.6
31–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	ND	40	2.3	3.6

[a] *Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements.*
[b] *The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.*
[c] *Although silicon has not been shown to cause adverse effects in humans, there is no justification for adding silicon to supplements.*
[d] *Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding vanadium to food and vanadium supplements should be used with caution. The UL is based on adverse effects in laboratory animals and this data could be used to set a UL for adults but not children and adolescents.*
[e] *ND, not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.*

Note: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Vitamins (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Vitamin A (μg/day)[a]	Vitamin C (mg/day)	Vitamin D (μg/day)[b,c]	Vitamin E (mg/day)[d]	Vitamin K (μg/day)	Thiamin (mg/day)	Riboflavin (mg/day)	Niacin (mg/day)[e]	Vitamin B_6 (mg/day)	Folate (μg/day)[f]	Vitamin B_{12} (μg/day)	Pantothenic Acid (mg/day)	Biotin (μg/day)	Choline (mg/day)[g]
Infants														
0–6 months	400*	40*	10	4*	2.0*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
6–12 months	500*	50*	10	5*	2.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
Children														
1–3 years	**300**	**15**	**15**	**6**	30*	**0.5**	**0.5**	**6**	**0.5**	**150**	**0.9**	2*	8*	200*
4–8 years	**400**	**25**	**15**	**7**	55*	**0.6**	**0.6**	**8**	**0.6**	**200**	**1.2**	3*	12*	250*
Males														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**900**	**75**	**15**	**15**	75*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	25*	550*
19–30 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
31–50 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
51–70 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
> 70 years	**900**	**90**	**20**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
Females														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**700**	**65**	**15**	**15**	75*	**1.0**	**1.0**	**14**	**1.2**	**400**[i]	**2.4**	5*	25*	400*
19–30 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
31–50 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
51–70 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
> 70 years	**700**	**75**	**20**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
Pregnancy														
14–18 years	**750**	**80**	**15**	**15**	75*	**1.4**	**1.4**	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
19–30 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
31–50 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[j]	**2.6**	6*	30*	450*
Lactation														
14–18 years	**1200**	**115**	**15**	**19**	75*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
19–30 years	**1300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
31–50 years	**1300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*

[a] *As retinol activity equivalents (RAEs): 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE.*

[b] *As cholecalciferol: 1 μg cholecalciferol = 40 IU vitamin D.*

[c] *Under the assumption of minimal sunlight.*

[d] *As α-tocopherol: α-Tocopherol includes RRR-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the 2R-stereoisomeric forms of α-tocopherol (RRR-, RSR-, RRS-, and RSS-α-tocopherol) that occur in fortified foods and supplements. It does not include the 2S-stereoisomeric forms of α-tocopherol (SRR-, SSR-, SRS-, and SSS-α-tocopherol), also found in fortified foods and supplements.*

[e] *As niacin equivalents (NE): 1 mg of niacin = 60 mg of tryptophan; 0–6 months = preformed niacin (not NE).*

[f] *As dietary folate equivalents (DFE). 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach.*

[g] *Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.*

[h] *Because 10–30% of older people may malabsorb food-bound B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with B_{12} or a supplement containing B_{12}.*

[i] *In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 μg from supplements or fortified foods in addition to intake of food folate from a varied diet.*

[j] *It is assumed that women will continue consuming 400 μg from supplements or fortified food until their pregnancy is confirmed and they enter prenatal care, which ordinarily occurs after the end of the periconceptional period—the critical time for formation of the neural tube.*

Note: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents from being able to specify with confidence the percentage of individuals covered by this intake.

Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Calcium (mg/day)	Chromium (µg/day)	Copper (µg/day)	Fluoride (mg/day)	Iodine (µg/day)	Iron (mg/day)	Magnesium (mg/day)	Manganese (mg/day)	Molybdenum (µg/day)	Phosphorus (mg/day)	Selenium (µg/day)	Zinc (mg/day)	Potassium (g/day)	Sodium (g/day)	Chloride (g/day)
Infants															
0–6 months	200*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	0.4*	0.12*	0.18*
6–12 months	260*	5.5*	220*	0.5*	130*	**11**	75*	0.6*	3*	275*	20*	**3**	0.7*	0.37*	0.57*
Children															
1–3 years	**700**	11*	**340**	0.7*	**90**	**7**	**80**	1.2*	**17**	**460**	**20**	**3**	3.0*	1.0*	1.5*
4–8 years	**1000**	15*	**440**	1*	**90**	**10**	**130**	1.5*	**22**	**500**	**30**	**5**	3.8*	1.2*	1.9*
Males															
9–13 years	**1300**	25*	**700**	2*	**120**	**8**	**240**	1.9*	**34**	**1250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1300**	35*	**890**	3*	**150**	**11**	**410**	2.2*	**43**	**1250**	**55**	**11**	4.7*	1.5*	2.3*
19–30 years	**1000**	35*	**900**	4*	**150**	**8**	**400**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
31–50 years	**1000**	35*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
51–70 years	**1000**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.3*	2.0*
>70 years	**1200**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.2*	1.8*
Females															
9–13 years	**1300**	21*	**700**	2*	**120**	**8**	**240**	1.6*	**34**	**1250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1300**	24*	**890**	3*	**150**	**15**	**360**	1.6*	**43**	**1250**	**55**	**9**	4.7*	1.5*	2.3*
19–30 years	**1000**	25*	**900**	3*	**150**	**18**	**310**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
31–50 years	**1000**	25*	**900**	3*	**150**	**18**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
51–70 years	**1200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.3*	2.0*
>70 years	**1200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.2*	1.8*
Pregnancy															
14–18 years	**1300**	29*	**1000**	3*	**220**	**27**	**400**	2.0*	**50**	**1250**	**60**	**12**	4.7*	1.5*	2.3*
19–30 years	**1000**	30*	**1000**	3*	**220**	**27**	**350**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
31–50 years	**1000**	30*	**1000**	3*	**220**	**27**	**360**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
Lactation															
14–18 years	**1300**	44*	**1300**	3*	**290**	**10**	**360**	2.6*	**50**	**1250**	**70**	**13**	5.1*	1.5*	2.3*
19–30 years	**1000**	45*	**1300**	3*	**290**	**9**	**310**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*
31–50 years	**1000**	45*	**1300**	3*	**290**	**9**	**320**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*

Note: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents from being able to specify with confidence the percentage of individuals covered by this intake.
Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); and Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Total Water and Macronutrients (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Total Water[a] (L/day)	Carbohydrate (g/day)	Total Fiber (g/day)	Fat (g/day)	Linoleic Acid (g/day)	α-Linolenic Acid (g/day)	Protein[b] (g/day)
Infants							
0–6 months	0.7*	60*	ND	31*	4.4*	0.5*	9.1*
6–12 months	0.8*	95*	ND	30*	4.6*	0.5*	**11.0**
Children							
1–3 years	1.3*	**130**	19*	ND[c]	7*	0.7*	**13**
4–8 years	1.7*	**130**	25*	ND	10*	0.9*	**19**
Males							
9–13 years	2.4*	**130**	31*	ND	12*	1.2*	**34**
14–18 years	3.3*	**130**	38*	ND	16*	1.6*	**52**
19–30 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
31–50 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
51–70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
>70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
Females							
9–13 years	2.1*	**130**	26*	ND	10*	1.0*	**34**
14–18 years	2.3*	**130**	26*	ND	11*	1.1*	**46**
19–30 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
31–50 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
51–70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
>70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
Pregnancy							
14–18 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
19–30 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
31–50 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
Lactation							
14–18	3.8*	**210**	29*	ND	13*	1.3*	**71**
19–30 years	3.8*	**210**	29*	ND	13*	1.3*	**71**
31–50 years	3.8*	**210**	29*	ND	13*	1.3*	**71**

[a]Total *water includes all water contained in food, beverages, and drinking water.*
[b]*Based on gram protein per kg of body weight for the reference body weight, for example, for adults 0.8 g/kg body weight for the reference body weight.*
[c]*ND, not determined.*
Note: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDA) in **bold type** and Adequate Intakes (AI) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevents from being able to specify with confidence the percentage of individuals covered by this intake.
Sources: From Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002–2005) and Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005). The report may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Acceptable Macronutrient Distribution Ranges (Food and Nutrition Board, Institute of Medicine, National Academies)

Macronutrient	Range (Percent of Energy)		
	Children, 1–3 years	Children, 4–18 years	Adults
Fat	30–40	25–35	20–35
n-6 Polyunsaturated fatty acids[a] (linoleic acid)	5–10	5–10	5–10
n-3 Polyunsaturated fatty acids[a] (α-linolenic acid)	0.6–1.2	0.6–1.2	0.6–1.2
Carbohydrate	45–65	45–65	45–65
Protein	5–20	10–30	10–35

[a]*Approximately 10% of the total can come from longer-chain* n-*3 or* n-*6 fatty acids.*
Source: From Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002–2005). The report may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Acceptable Macronutrient Distribution Ranges (Food and Nutrition Board, Institute of Medicine, National Academies)

Macronutrient	Recommendation
Dietary cholesterol	As low as possible while consuming a nutritionally adequate diet
Trans fatty acids	As low as possible while consuming a nutritionally adequate diet
Saturated fatty acids	As low as possible while consuming a nutritionally adequate diet
Added sugars[a]	Limit to no more than 25% of total energy

[a]*Not a recommended intake. A daily intake of added sugars that individuals should aim for to achieve a healthful diet was not set.*
Source: From Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (2002–2005). The report may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Vitamins (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Vitamin A (μg/day)[a]	Vitamin C (mg/day)	Vitamin D (μg/day)	Vitamin E (mg/day)[b,c]	Vitamin K	Thiamin	Riboflavin	Niacin (mg/day)[c]	Vitamin B_6 (mg/day)	Folate (μg/day)[c]	Vitamin B_{12}	Pantothenic Acid	Biotin	Choline (g/day)	Carotenoids[d]
Infants															
0–6 months	600	ND[e]	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6–12 months	600	ND	38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Children															
1–3 years	600	400	63	200	ND	ND	ND	10	30	300	ND	ND	ND	1.0	ND
4–8 years	900	650	75	300	ND	ND	ND	15	40	400	ND	ND	ND	1.0	ND
Males															
9–13 years	1700	1200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
51–70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
>70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Females															
9–13 years	1700	1200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
51–70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
>70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Pregnancy															
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Lactation															
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND

[a] *As preformed vitamin A only.*
[b] *As α-tocopherol: applies to any form of supplemental α-tocopherol.*
[c] *The ULs for vitamin E, niacin, and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.*
[d] *β-Carotene supplements are advised only to serve as a provitamin A source for individuals at risk of vitamin A deficiency.*
[e] *ND, not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.*

Note: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels, Elements (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Arsenic[a]	Boron (mg/day)	Calcium (mg/day)	Chromium	Copper (μg/day)	Fluoride (mg/day)	Iodine (μg/day)	Iron (mg/day)	Magnesium (mg/day)[b]	Manganese (mg/day)	Molybdenum (μg/day)	Nickel (mg/day)	Phosphorus (g/day)	Selenium (μg/day)	Silicon[c]	Vanadium (mg/day)[d]	Zinc (mg/day)	Sodium (g/day)	Chloride (g/day)
Infants																			
0–6 months	ND[e]	ND	1000	ND	ND	0.7	ND	40	ND	ND	ND	ND	ND	45	ND	ND	4	ND	ND
6–12 months	ND	ND	1500	ND	ND	0.9	ND	40	ND	ND	ND	ND	ND	60	ND	ND	5	ND	ND
Children																			
1–3 years	ND	3	2500	ND	1000	1.3	200	40	65	2	300	0.2	3	90	ND	ND	7	1.5	2.3
4–8 years	ND	6	2500	ND	3000	2.2	300	40	110	3	600	0.3	3	150	ND	ND	12	1.9	2.9
Males																			
9–13 years	ND	11	3000	ND	5000	10	600	40	350	6	1100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	3	400	ND	1.8	40	2.3	3.6
Females																			
9–13 years	ND	11	3000	ND	5000	10	600	40	350	6	1100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2000	ND	10,000	10	1100	45	350	11	2000	1.0	3	400	ND	1.8	40	2.3	3.6
Pregnancy																			
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	3.5	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	3.5	400	ND	ND	40	2.3	3.6
31–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	3.5	400	ND	ND	40	2.3	3.6
Lactation																			
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	ND	40	2.3	3.6
31–50 years	ND	20	2500	ND	10,000	10	1100	45	350	11	2000	1.0	4	400	ND	ND	40	2.3	3.6

[a] *Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements.*
[b] *The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.*
[c] *Although silicon has not been shown to cause adverse effects in humans, there is no justification for adding silicon to supplements.*
[d] *Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding vanadium to food and vanadium supplements should be used with caution. The UL is based on adverse effects in laboratory animals and this data could be used to set a UL for adults but not children and adolescents.*
[e] *ND, not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.*

Note: A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

Sources: From Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

Dietary Reference Intakes for Calcium and Vitamin D (Food and Nutrition Board, Institute of Medicine, National Academies)

Life Stage Group	Estimated Average Requirement (mg/day)	Calcium Recommended Dietary Allowance (mg/day)	Upper Level Intake (mg/day)	Vitamin D Estimated Average Requirement (IU/day)	Recommended Dietary Allowance (IU/day)	Upper Level Intake (IU/day)
Infants 0–6 months	*	*	1.000	**	**	1.000
Infants 6–12 months	*	*	1.500	**	**	1.500
1–3 years old	500	700	2.500	400	600	2.500
4–8 years old	800	1.000	2.500	400	600	3.000
9–13 years old	1.100	1.300	3.000	400	600	4.000
14–18 years old	1.100	1.300	3.000	400	600	4.000
19–30 years old	800	1.000	2.500	400	600	4.000
31–50 years old	800	1.000	2.500	400	600	4.000
51–70 year old males	800	1.000	2.000	400	600	4.000
51–70 year old females	1.000	1.200	2.000	400	600	4.000
>70 years old	1.000	1.200	2.000	400	800	4.000
14–18 years old, pregnant/lactating	1.100	1.300	3.000	400	600	4.000
19–50 years old, pregnant/lactating	800	1.000	2.500	400	600	4.000

*For infants: Adequate Intake is 200 mg/day for 0–6 months of age and 260 mg/day for 6–12 months of age.
**For infants: Adequate Intake is 400 IU/day for 0–6 months of age and 400 IU/day for 6–12 months of age.

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