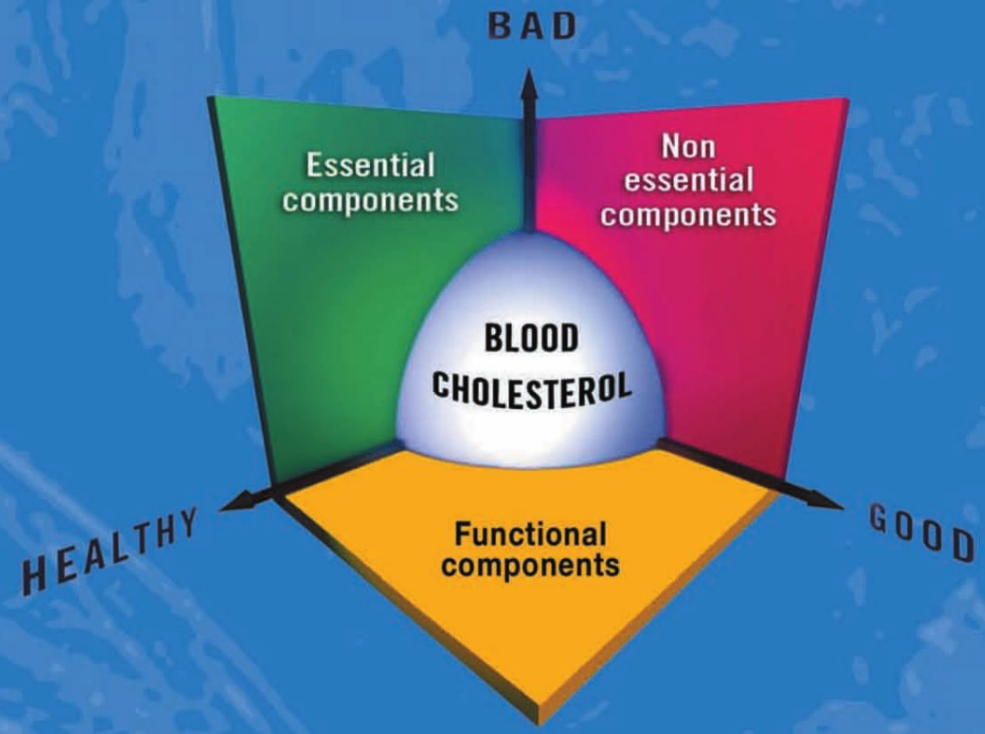
Wild-Type Food in Health Promotion and Disease Prevention

The Columbus Concept

Edited by

Fabien De Meester, PhD
Ronald Ross Watson, PhD



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The Columbus Concept

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Preface

For most of us, it requires some effort to dare realize how wrong we were: Blood cholesterol was never a problem. Blood vessels were the issue; this fact is scientifically, clinically, and epidemiologically established. *Wild-Type Food in Health Promotion and Disease Prevention: The Columbus Concept* will bring this evidence into the public domain. It is essential to make a "U-turn" in the way contemporary medicine approaches ill-health diseases. Early prevention of primary risk factors is a far more promising approach compared with late-acute treatment of secondary risk factors in reducing the cost burden of public health, not to mention the expected benefits of the former versus the latter regarding quality of life.

The leading market concept behind the rehabilitation of dietary/blood cholesterol is called "Columbus." Obviously, it borrows the significance of the name from the time when the earth was proven round rather than flat, and a dramatic leap was made in the way people understood the world they lived in. However, facts are not enough to catalyze change. There must be a will, fueled by the hope of an opportunity or the fear of a threat for those involved. As far as the cholesterol–health relationship is concerned, the opportunity in terms of economic development and the threat in terms of civil responsibilities become more sensible every day that science, industry, and legislation progress.

Wild-Type Food in Health Promotion and Disease Prevention: The Columbus Concept is divided into five major parts of equal importance. Part I identifies the missing essential ratio/proportion of competing essential fatty acids in the human diet and relates them to the upsurge of modern chronic diseases and associated health care costs, with special emphasis on coronary heart disease. Fabien De Meester explains the scientific basis of the Columbus Concept and the evidence supporting the rehabilitation of dietary/blood cholesterol. Tomohito Hamazaki and Harumi Okuyama show that blood cholesterol is a necessity for a population to maintain healthy blood vessels (Japan), while Gal Dubnov-Raz and Elliot M. Berry identify the medical consequences of a high proportion of omega-6 HUFAs in blood vessels and peripheral tissues in a population (Israel) whose dietary pattern has been characterized by high to extremely high omega-6:omega-3 ratios. Jing Xuan Kang reports on the ratio and proportion of omega-6 to omega-3 fatty acids naturally found in genetically modified organisms that are allowed to desaturate the former to the latter and, so doing, validate the wild-type hypothesis at the molecular level. William E. Lands describes the biochemical and mathematical relationships that have been established between dietary and tissue essential fatty acid ratio/proportion to conclude that our health is closely associated with our dietary choices in terms of lipid (essential fatty acid) make-up. Jonathan D. Belsey takes these conclusions and reviews the results of major prospective clinical studies to estimate the potential public health savings that would result from the implementation of the Columbus Concept in high-risk

cardiovascular populations only. Finally, S. Boyd Eaton closes the Part I with a discussion on the world perspective of wild-type diet in health promotion.

Part II concentrates on the 10% (in terms of their contribution to the daily energy intake) essential ingredients (amino acids, fatty acids, vitamins, and minerals) in our daily diet that make our blood vessels healthy and resistant. The first seven chapters of Part II use eggs as a model. Fabien De Meester describes the wild-type egg as an ideal candidate for the future establishment of a WHO reference pattern for dietary fatty acids in humans. Basant K. Puri and Jonathan P. Stannard report on the essentiality of eicosapentenoic acid (once disputed because it competes against AA) in human milk and the efficiency of the wild-type egg in supplying this essential omega-3 fatty acid to breast milk through the mother's diet. Peter F. Surai and Brian K. Speake emphasize the changes that have occurred in the composition of eggs as a result of the domestication of the birds. With their collaborators, Tigran T. Papazyan and Nick H. C. Sparks, they explain how we can, and why we should, return modern eggs to their standard in the wild. Niva Shapira describes a lipid endothelial and inflammatory hypothesis that explains how egg composition can affect cardiovascular disease risk. Finally, Gita Cherian and Ricardo Ayerza (h) illustrate ways to produce and characterize eggs with increased levels of omega-3 fatty acids obtained by feeding layers of flax and chia seeds, respectively. The remaining eight chapters relate to the physiological/metabolic properties of omega-3 HUFAs, taking fish oil as the complementary source to wild land-based plant and animal omega-3 sources. Adrian S. Dobs and Daniel Edelstein start with an overview of fish oil and health promotion and introduce the basis for a series of leading articles on the established relationship between dietary omega-3 fatty acids and the occurrence of ill-health diseases. Michel de Lorgeril and Patricia Salen present the extended amount of accumulated evidence on the involvement of omega-3 fatty acids in regulating cardiovascular health and, in turn, their potential use in primary prevention of coronary heart disease. Vijaya Juturu and James J. Gormley take the discussion forward to describe how the absence/imbalance of essential omega-3 fatty acids modulates insulin resistance, while Ram B. Singh et al. extend that discussion to involve the brain, and propose a modern dietary therapy for the metabolic syndrome based on an appropriate balance of dietary essential fats. Erin M. O'Connell, Patricia D. Schley, and Catherine J. Field show how, interestingly, omega-3 fatty acids are mediating immunomodulation and cancer resistance. Mental health and diseases associated with imbalance in dietary essential fatty acids are reviewed by Abolghassem Djazayery and Shima Jazayery, while Sheila Sedig and Ronald Ross Watson review the role of omega-3 fatty acids in prevention of aged-related macular degeneration. The last two chapters of Part II introduce two non-lipid essential ingredients of the diet that contribute synergistically with omega-3 fatty acids to the maintenance of healthy blood vessels and peripheral tissues, including bones. Brandon Lewis reviews the essentiality of the natural carotenoid lutein in promoting eye and skin health, and Rainy Dawn Warf and Ronald Ross Watson presents evidence for calcium in protecting teeth against degenerative dental caries.

Part III introduces the functional part of the diet that can bring about health benefits in terms of maintaining healthy blood vessels. Dietary functional components include dietary ingredients (phytochemicals, pro-, pre-, synbiotics) and other lifestyle factors (physical activity, spiritualism). They usually do not contribute to an appreciable extent

to the daily energy intake. It will become obvious to the reader that some chapters treat both essential and functional aspects of the human diet and lifestyle. Because the authors were not consigned to limit themselves to the description of the one (essential) or the other (functional) aspect independently, those chapters treating the two aspects simultaneously were collected in Part III.

Peter F. Surai, Ambrose J. Spinnler Benadé, and Brian K. Speake review the distribution of natural antioxidants in land- and marine-based food, and explain how such distribution is important to maintain in modern food, especially if the latter are rebalanced in wild essential fatty acids. Ricardo Ayerza (h) and Wayne E. Coates introduce the tale of an ancient crop of Latin America that came back to life thanks to the reviving interest towards wild food rich in omega-3 fatty acids and essential/functional antioxidants. Chia seed (*Salvia hispanica*) is presented as an ideal vector of land-based omega-3 fatty acids to modern human's diet. Other phytochemicals and their influence on ill-health diseases are reviewed by Madhuri Vemuri, Darshan S. Kelley, and Kent L. Erickson in their article on foods rich in polyphenols; by Tirang R. Neyestani in his report on evidence of a relationship between the presence of polyphenols in the diet and the possible modulation of immunity; by Sherma Zibadi, Douglas F. Larson, and Ronald Ross Watson in a review on the influence of flavonoids on heart failure; and by Simin Bolourch-Vaghefi and Paula Inserra in chapters on the functional properties of natural carotenoids—lycopene—in preventing modern diseases in general (Vaghefi), and prostate cancer in particular (Inserra). Reading through Part III, it appears that functional ingredients of the diet need not be absorbed to a significant level to be active. Their action at the food/blood interface (intestine) may well be their primary site of action.

Part IV presents the health benefits of wild-type foods as found naturally in different cultures around the globe, starting with Francesco Visioli, Franca Marangoni, and Claudio Galli with their review on local wild foods in the Mediterranean countries, and Manuel J. Castilló Garzón on the effects on plasma lipids as a result of the recent changes observed in the Spanish-Mediterranean diet. Nahla Hwalla and Dalia Tannous Dit El Khoury give us an introduction to the Middle East with the traditional Lebanese diet and its recognized health effects, while Abolghassem Djazayery and Shima Jazayery collect information for us on the health status of Iranian people relative to their choice in terms of nutritional lipids and fatty acids. Finally, Buncha Ooraikul, Anchalee Sirichote, and Sunisa Siripongvuthikorn report on the traditional and modern diet/health relationship in South East Asia, taking Thailand as their native target country. This part supports the theory that each region around the world has the potential to maintain wild dietary standards compatible with human genome, long-term homeostatis, and health, extended to blood vessels and peripheral tissues.

The fifth and final part is an essay toward bringing the Columbus Concept to the market in a world driven by economic and financial factors that provides little, if any, support to all kinds of preventive approaches to health and medicine. Luc Coucke starts by describing how different modern wild-type agriculture will have to be compared to today's modern standards. Alfredo Nasiff-Hadad and Jiménez-Acosta Santa Magaly describe a way to definitively test and prove the wild-type diet and lifestyle hypothesis in a country (Cuba) where wild-type standards could still be established without the burden of fighting *per se* against economic and financial pressure. Finally, Ambrose J.

Spinnler Benadé concludes with his proposal to test for the Columbus Paleolithic hypothesis on plasma lipoprotein composition, metabolism, and atherosclerosis in a species closely genetically related to man, the vervet monkey.

It is obvious that the Columbus Concept is in its early stages of market development. Given the general public confusion with the cholesterol myth, it is evident that only a step-by-step reconstruction and the rehabilitation of a Dietary Food Guide based on Paleolithic lipid standards can be thought of as a leading pattern to true public health and quality of life. Knowing what is at stake, there is no doubt that it is a good investment.

Fabien De Meester
Ronald Ross Watson

Acknowledgments

Wild-Type Food in Health Promotion and Disease Prevention: The Columbus Concept is the result of 10 years of teamwork by a team whose members are establishing a value chain organized to take a sound scientific concept to a market reality. Those members are not part of a single company or institution; rather, they are independent members taking part in the shaping and building up of a science-led business concept, i.e., the Dietary Food Guide based on Paleolithic Lipid Standards, which, among other things, advocates the normal functioning of diet/blood ingredients and the rehabilitation of dietary/blood cholesterol. On the market, the concept is branded as "Columbus."*

The members belong to a large web of public and private organizations, with expertise extending from science and technology to marketing, legal matters, economics, and finance. The organization of the Consortium has been described in the Common Statement of the First International Congress on the Columbus Concept (ICCC) that took place in Washington, D.C., in October 2002. Each year, an ICCC is organized to review progress in bringing the science of the Columbus Concept to the market. In 2006, the event coincided with ISSFAL in Cairns, Australia, where a concurrent session on dietary PUFAs and cholesterol was organized and sponsored by Columbus (see www.columbus-concept.com for regularly updated information).

To all contributors, Dr. De Meester wishes to extend his most sincere gratitude for achieving such progress as a team, and promises to do what it takes for this concept to establish itself as the reference in human nutrition and preventive medicine throughout the industry.

Dr. Watson acknowledges the research support for the past few decades from Wallace Research Foundation, led by H. B. and Joceyln Wallace supporting studies using dietary supplements, eggs, foods, and bioactive extracts in health promotion by preventing heart disease and immune dysfunction. This led to his role as a coeditor of this book.

A special appreciation is due to Bethany L. Stevens, who worked on this book from the beginning as the editorial assistant. She diligently supported the editors, publisher, and especially the authors in making their contributions the excellent chapters found in this volume. Her many hours of day-to-day communication are therefore much appreciated by the editors.

* In the United States, Columbus products appear under the brand "Christopher."

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I

The Columbus Concept: Scope and Limitation

1 Wild-Type Land-Based Food in Health Promotion and Disease Prevention

The LDL-CC:HDL-CC Model

Fabien De Meester

Abstract

The Columbus Concept (CC) stands for a return of α-linolenic acid—herein referred to as wild- or game-type, land-based fatty acid—into the feed ration of land-based, bred animals to such an extent that their body fat (white adipose tissue) and triglycerides (TGs) exhibit a balanced ratio of essential fatty acids (EFAs) (i.e., ω6:ω3-EFAs/TGs = 1:1), characteristic of body fat in wild animals or game. This return to the standard in the wild translates into a substantial reduction in highly unsaturated omega-6 fatty acids and a moderate and variable increase in highly unsaturated omega-3 fatty acids in the peripheral tissues of these domesticated, land-based animals or livestock. In other words, their meat total lipids (TLs) exhibit a proportion (%) of conditionally-essential highly unsaturated fatty acids (HUFAs) in favor of the omega-3 species (i.e. ω6/(ω6 + ω3)-HUFAs/TLs = 25%), characteristic of meat lipids in wild, land-based animals and game. The ω6:ω3-EFAs/TGs = 1:1 ratio is also a reference for the design of composite, plant-derived table oils and fats, as these represent other important sources of energy in the modern human diet.

Particular attention is given to the antioxidant content (vegetables, fruits) of such foods.

Finally, a new and simple mathematical equation is proposed that provides a basis for accurate prediction of individual risk for CHD based on the measurement of two blood parameters: total blood cholesterol level (TC) and proportion of omega-6 HUFAs in blood total HUFAs (i.e., ω6/(ω6 + ω3)-HUFAs/TLs), the former being phenotype-driven, the latter in direct relationship with peripheral-tissue HUFAs-composition.

Key Words: Wild- or game-type land-based food; omega-6 and omega-3 fatty acids; LDL-CC:HDL-CC; degenerative diseases.

1. INTRODUCTION

The theory that dietary cholesterol and dietary saturated fats are causal factors for elevated blood cholesterol and cardiovascular disease has successfully influenced our dietary habits—giving eggs, milk, and meat questionable nutritional status. It is now known that blood cholesterol is not a primary risk factor for coronary heart disease and that the diet–heart hypothesis holds true greatly to the extent that our dietary lipids in general, the essential fatty acids in particular, have deviated from their original make-up. Even more significant is that high blood cholesterol might be a sign of health and longevity.

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Table 1
Essential Fatty Acids (as % of Total Fatty Acids) in Adipose Fats of Wild Pig and Antelope

Adipose fat	*Pig*	*Antelope*
ω6-linoleic	17	4.5
ω3-linolenic	17	4.0
Total	34	8.5
ω6:ω3	±1	±1

It is time to re-establish the land-based "Golden Game Standards," defined *(1)* as LA:ALA = 1:1 in the TGs of body fat in livestock and *(2)* as ω6/(ω6 + ω3)-HUFAs/TLs = 25% in the TLs of their meat, and to test them in large retrospective and prospective epidemiological studies.

2. FATS IN THE PALEOLITHIC LAND-BASED DIET

Wild animals, plants, and fruits were the major food sources available to our hunter-gatherer, unhumanized Paleolithic ancestors. Hunting is still practiced as a sport in some parts of the world, and contemporary scientists have measured the ω6:ω3-EFAs/TGs ratio that persists in untamed land environments such as the African Savannah. They discovered that adipose fats, which account for ±95% of total fat in land-based animals, differ from species to species by their respective content in essential fatty acids (*see* Table 1; 34% in monogastric pig vs 8.5% in ruminant antelope), but that their essential ω6:ω3-EFAs/TGs ratio consistently nears equilibrium *(1,2)*. More recent studies on wild animals confirmed original observations by Crawford and Sinclair *(3)*.

During human evolution, modern cereals and grains were scarce, and the food available to preagricultural humans was essentially wild and lean (e.g., meat, fish, leafy greens and plants, fruits, nuts, berries) and loaded with antioxidant vitamins and minerals. Under such Paleolithic-type, environmental conditions, it is generally estimated that the white adipose tissue of wild animals and game accounted for the major source of land-based dietary lipids and that the average ω6:ω3-EFAs/TGs ratio in that land-based diet was therefore close to 1:1 *(3,4)*.

3. FATS IN THE MODERN LAND-BASED DIET

It is obvious that agribusiness (grain and cereal production) and food technology (fat and oil extraction) have dramatically changed the pattern of nutrients and lifestyles in the human regimen. Energy-dense, fat-rich foods and sedentary lifestyles have become standard. However, all things remaining equal in terms of essential principles *(5)* (i.e., energy intake = energy expenditure, proportionality, variety and moderation) modernization has induced a single dramatic change in the human regimen which is the way essential nutrients are distributed. Basically, modern foods are loaded with omega-6 fats and are moderately to largely deficient in omega-3 fats, antioxidant vitamins, and minerals. This shift in paradigm is a logical or induced consequence of the modern turn from the wild, versatile diet, based on greens and game, to the modern, easy-to-access diet, based on grains and livestock.

It has been estimated that the modern Western diet is largely deficient in omega-3 essential fatty acids with a ratio of omega-6 to omega-3 ranging from 20:1 to 10:1, instead of 1:1 as in the Paleolithic diet *(3)*.

4. FATS IN THE COLUMBUS DIET

Since the Paleolithic era, and because the time period under consideration is only a few thousand years, humanity as a phenotype has evolved thanks to environmental and developmental changes rather than genetic modification. The evolving human phenotype, therefore, interacts with the same genome as did the hunter-gatherer phenotype. Over the last 150 to 50 yr of industrial revolution and modernization, however, a major change has occurred in the human diet: essential fats and antioxidant vitamins and minerals have been manipulated. Essential fats are those that cannot be produced or exchanged within the human body; they must be extracted from food and are involved in human gene expression and overall homeostasis. There are two types: the omega-6 (ω6) and the omega-3 (ω3); these two types compete against each other in human, fatty-acid, biological pathways. It is therefore interesting to evaluate and test the most probable dietary ratios that existed between the two types of essential and conditionally-essential fatty acids at the inception of our ancestor's genome, for there is no obvious reason for a necessary change since then.

The Columbus Concept recognizes that it is both essential and urgent to test for a return to wild-type, balanced essential fatty acid ratios (ω6:ω3-EFAs/TGs = 1:1 $\Rightarrow$ ω6/(ω6 + ω3)-HUFAs/TLs = 25%) in the human diet associated with the delivery of appropriate amounts of essential and functional antioxidants (e.g, vitamins C and E, selenium, xanthophylls, phytochemicals) from grains, cereals, fruits, legumes, vegetables, meat, milk, and eggs. From an industrial perspective, the Columbus Concept stands for a "return to wild-type, land-based foods based on modern economically viable agribusiness standards," which involves a second agribusiness revolution (*see* Fig.1). This revolution encompasses large-scale production of naturally-occurring, omega-3 rich seeds (e.g., colza, flax, chia, perilla), low-fat cereals (e.g., wheat, rice), and genetically selected and/or modified omega-3 versions of naturally-occurring omega-6 rich grains (e.g., sunflower, corn, cotton, soy) to the extent that *(1)* modern intensive animal husbandry can be maintained on feed lipid patterns reminiscent to those of grass and greens and *(2)* the human diet can be maintained on Golden Game Standards similar to that of our Paleolithic ancestors. The end result could eventually be an unchanged food supply in terms of quantity, variety, and stability but with improved or more human-gene-compliant essential-fatty-acid, antioxidant-vitamin, and mineral patterns.

5. CHOLESTEROL IN THE COLUMBUS DIET

Dietary cholesterol, dietary saturated fats, and blood cholesterol have been hot issues over the last 40 to 50 yr and still are. Terms such as "bad" and "good" cholesterols are deeply imprinted in the subconscious of the general population. HCPs are used to describe the apparent atherogenicity of high- vs low-LDL-C:HDL-C ratios, respectively. Most if not all means associated with the human health regimen—nutritional, medical, environmental, spiritual—tested so far for the treatment and/or the prevention of cardiovascular disease have targeted the LDL-C:HDL-C ratio. As shown in Fig. 2,

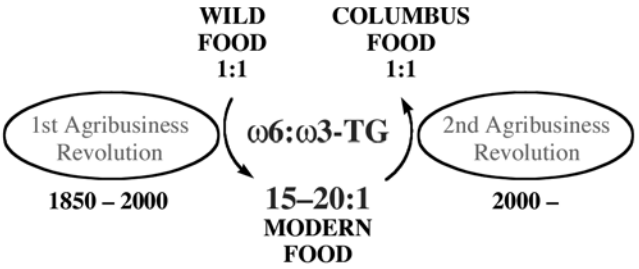

Fig. 1. The Columbus Concept and its vision.Wild food to Modern food to Columbus® food as led by the 1st and 2nd agribusiness revolutions respectively. Modern crop production and processing have supported, in terms of energy supply, an outgrowth of the world population, but they have concomitantly induced drastic changes in the essential dietary fatty acids and associated antioxidant supplies in the human diet. Besides its obvious blessing, one dramatic drawback of the paradigm shift in food supplies—from wild to modern—are the so-called modern degenerative diseases becoming ever more endemic in most developed and developing countries. Scientific, clinical, and epidemiological data support the view that this drawback is in large part induced by the moderate to high ω6:ω3-EFAs/TGs ratios in modern foods from plant and animal origins and therefore in human tissues. The Columbus Concept describes a second possible agribusiness revolution that sustains a return to wild-type food (ω6:ω3-EFAs/TGs = 1:1 ⇒ ω6/(ω6 + ω3)-HUFAs/TLs = 25%) based on modern, economically viable agribusiness standards.

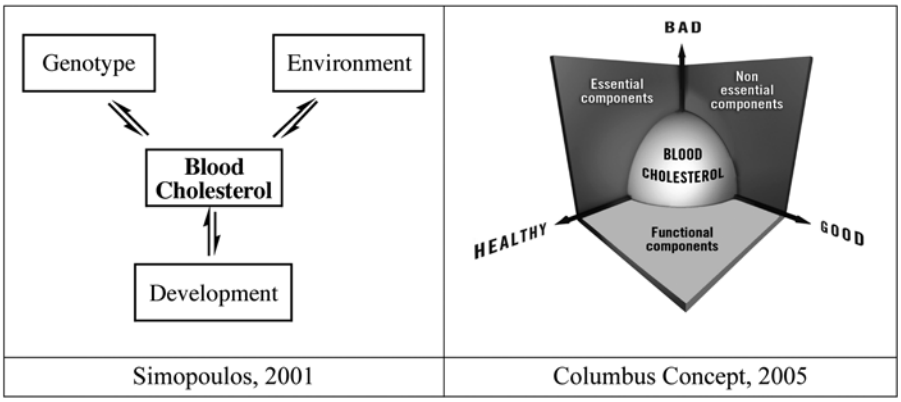

Fig. 2. The Columbus Concept and its 3D representation of blood lipoprotein behaviors. "Bad" LDL-C, "good" HDL-C, and "healthy" LDL-CC:HDL-CC ratios. Blood cholesterol depends on genetic factors, health regimen, and development. As any other phenotype-driven parameter, it varies with circumstances and age. As such, in no case can it be declared as an independent risk factor for cardiovascular or any other degenerative diseases. Actually, it can be a good thing provided it is associated with appropriate and balanced amounts of essential nutrients (fatty acids and antioxidants). The 3D representation of blood cholesterol in the Columbus Diet allows one to immediately identify the components (nonessential, functional, and essential) of the human regimen that may influence blood lipoprotein behavior in the vascular systems of the human body.

however, blood-cholesterol level (TC) and distribution (LDL:HDL ratio) are phenotype-driven and susceptible to various components of the human health regimen (i.e., nonessential, functional, and essential). Induced changes in blood-cholesterol-related parameters have in fact seldom rather than always shown positive results in the prevention and

treatment of cardiovascular disease (*see* ref. *6*, for a thorough review and critique of past, major clinical and epidemiological studies on the controversial dogmatic subject, the multitude of misleading interpretations and myths created, and the consequent developments thereof). Also, some more recent large epidemiological studies have clearly demonstrated that blood cholesterol is not an independent risk factor in the development of cardiovascular disease and that high blood cholesterol might in fact be a marker for longevity in the elderly *(7,8)*. Within this framework of maintained confusion—itself seeded largely by the spread of misunderstanding and sometimes conflicting interests—the Columbus Concept works toward a truly scientific rehabilitation of the long-term health benefits of blood lipoprotein patterns associated with, or characterized by, appropriate and balanced amounts of essential dietary nutrients (essential fatty acids and antioxidant vitamins and minerals). Hereafter, this ideal individual blood lipoprotein pattern is referred to as LDL-CC:HDL-CC, where CC stands for Columbus Concept (i.e., the individual phenotype-driven LDL-C:HDL-C ratio associated with ideal wild-type, diet-sensitive, essential-fatty-acid and antioxidant patterns [*see* Fig.2]).

The introduction of this concept of LDL-CC:HDL-CC ratio is important because it automatically leads to a need for re-interpreting past clinical and epidemiological studies in terms of dietary compliance with RDIs for essential nutrients, including omega-3 fatty acids and antioxidant vitamins and minerals.

Compared with the traditional 2D representation of "bad" and "good" LDL-C:HDL-C ratios that globalizes the influence of all components of the human regimen, it is seen that the proposed 3D representation allows one to refine the analysis to the three classes of components: the nonessential nutrients that represent ±90% of the DEI, the functional components that do not substantially contribute to the DEI, and the essential nutrients that account for ±10% of the DEI. It then becomes clear that a supposedly good LDL-C:HDL-C ratio can be atherogenic if some essential components of the regimen are not present, and, vice versa, a supposedly bad LDL-C:HDL-C ratio can be nonatherogenic if all the necessary essential components of the regimen are present. In the same representation, functional components appear potentially beneficial to human health, especially in the case of bodily deficiencies and/or higher sudden requirements in essential nutrients. Functional components can be anything from dietary (e.g., phytochemicals, pre- and probiotics) to behavioral (e.g., sport, relaxation, resting, chronobioethics), environmental (e.g., altitude, electromagnetism, weather conditions), spiritual (e.g., mind, ethic, soul), and other kinds.

Epidemiological studies tend to show that the innovative healthy cholesterol or LDL-CC:HDL-CC avenue is a more promising healthcare investment on the long term because higher blood cholesterol now tends to be recognized as an indicator of longevity in the elderly.

But what are these new species, LDL-CC and HDL-CC?

6. DEFINITION OF LDL-CC:HDL-CC

Lands has demonstrated that CHD-mortality in various populations around the world accurately reflects their blood-relative concentrations of long-chain omega-6 fatty acids (%Omega-6 HUFAs in total HUFAs or (ω6/(ω6 + ω3))-HUFAs/TLs) (*see* Fig. 3).

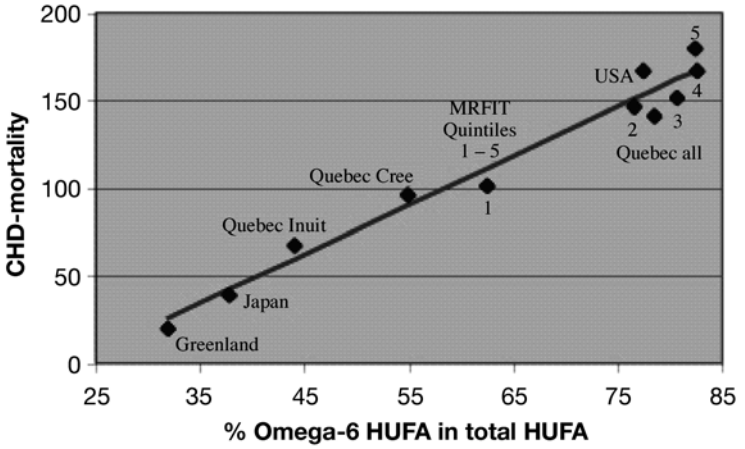

Fig. 3. Lands has demonstrated that there is an almost perfect correlation between the death toll per CHD (#/100,000/yr) in various populations around the world and their blood proportion of highly unsaturated omega-6 fatty acids relative to their blood-total concentration of highly unsaturated fatty acids (HUFAs): Greenlanders, Japanese, and Inuits have lower blood-relative concentrations of omega-6 HUFAs and present substantially lower rates of CHD-mortality than western populations characterized by high blood-relative concentrations of omega-6 HUFAs. The apparent absence of the influence of blood cholesterol on this (linear) relationship may result from the small (0–0.2%) contemplated scale of CHD-mortality recorded in a limited time period (1 yr.).

Linear extrapolation of the data shown in Fig. 3 leads to a 25% tissue-relative concentration of omega-6 HUFAs (as compared with 75–85% in western populations) as a blood-cholesterol-independent, immediate, potentially ideal protection against death from CHD, such as

$$\%\text{CHD Mortality} = 3 \times (\%\omega6/(\omega6 + \omega3)\text{-HUFAs/TLs} - 25)$$

The Lands diagram therefore tells us that the human tissue and therefore dietary essential-fatty-acid compositions quite accurately predict how likely humans will die from CHD. Clinical trials led in Britain, France, Italy, and India over the last 20 yr (1985–2002) have confirmed the wild-diet, cause-effect relationship—the reproducibility of the *immediate* health (cardiac) benefits related to the dietary intake of wild lipids (green-type ω3-EFA [ALA] and game-type ω3-HUFAs [EPA, DPA, DHA]) in high-risk patients (secondary prevention) traditionally fed omega-6-rich grain- and livestock-based modern, domesticated foods Table 2).

A meta-analysis of these clinical trials *(9)* has confirmed that a wild-type diet enriched in omega-3 fatty acids characteristic of greens and game does protect, complementarily and synergistically, against cardiovascular diseases to an extent where the risk of dying from a cardiovascular disease becomes a question of personal choice (*see* Fig. 4).

Based on such strong scientific evidence, we define LDL-CC and HDL-CC as the LDL-C and HDL-C species that coexist in a vascular environment defined by a blood total lipid composition that complies with the standard in the wild: $\omega6/(\omega6 + \omega3)$ – HUFAs/TLs = 25%.

Table 2
Results of Randomized Clinical Trials

Trial	*Length*	*No. of patients*	*Lipid source*	*Total death rate*	*Cardian death rate*	*Nonfatal cardiac events*
IHT	1 yr	500	GREENS (ω3-EFA)	–35%*	–45%**	–50%
LDHS	5 yr	605	GREENS (ω3-EFA)	–50%	–50%	–50%
DART	2 yr	2033	GAME (ω3-HUFAs)	–30%	–30%	–
GISSI	1 yr	11324	GAME (ω3-HUFAs)	–20%	–30%	–

Abbr: IHT: Indian Heart Trial *(10,11)*; LDHS: Lyon Diet Heart Study *(12,13)*; DART: The Diet and Reinfarction Trial *(14)*; GISSI: *(15,16)*.

Notes: *Borderline, non-significant; **Not provided, calculated by de Lorgeril.

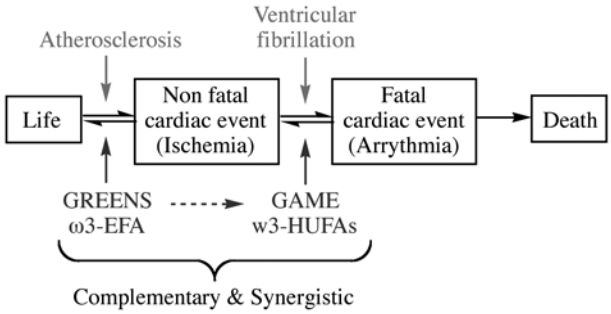

Fig. 4. Complementary and synergistic heart benefits of green-type (ALA) and game-type (EPA, DPA, DHA) fatty acids in the diet. Clinical trials tend to show that green-type fatty acid (ALA, C18:3 ω3) does reduce ischemic and arrhythmic events in secondary prevention, whereas long-chain, game-type fatty acids (EPA, C20:5 ω3; DPA, C22:5 ω3; DHA, C22:6 ω3) are more specific towards final-stage arrhythmia. Given that ALA and DHA can compete with LA and AA, on the one hand, and that both can be turned to EPA, on the other hand, one sees that green-type and game-type omega-3 fatty acids have complementary and synergistic health benefits within the human body, as reflected by the results of the clinical trials reported in Table 2.

7. AGRIBUSINESS STANDARDS UNDER THE COLUMBUS CONCEPT

Chloroplast-bearing grasses and greens are relatively low in fat (±1%), but proportionally rich in omega-3 fatty acids and antioxidants that protect them against sunshine irradiation. On the other hand, grains and cereals are energy-dense (e.g., rich in fats and/or carbohydrates) but proportionally poor in omega-3 fatty acids and antioxidants. Most grains and cereals are rich in omega-6 fatty acids. The Columbus Concept favors omega-3 rich seeds and grains, which when combined with low-fat cereals (wheat, rice) and natural antioxidants, provide composite animal feeds with fatty-acid patterns and oxidative stability similar to those of grasses and greens (Table 3).

Table 3
Omega-6:Omega-3 Ratio in Wild-Type Animal Feed and Leafy Vegetables

		MUFA	*PUFA*		
Vegetable plant	*SAFA*	*ω7 + ω9*	*ω6*	*ω3*	*ω6:ω3*
Cabbage, red	25	5	30	40	0.75
Columbus Feed	**12**	**18**	**25**	**45**	**0.58**
Parsley	18	3	26	54	0.48
Lettuce	18	3	17	44	0.38
Cabbage, white	18	8	15	58	0.26
Flax seeds	9	18	15	57	0.26
Cauliflower	22	15	13	50	0.26
Brussels sprouts	20	5	12	63	0.19
Spinach	12	3	8	52	0.16

Fatty acid composition of total lipid extracted from edible parts of the vegetable (i.e., roots, stems, leaves, flowers, as appropriate *[7]*).

Table 4
Antioxidants (A.O.) in Wild-Type Animal Feed and Leafy Vegetables

Antioxidants	*Derivatives*
Vitamin C	Ascorbic acid, esters, etc.
Vitamin E	Tocopherols, esters, etc.
Polyphenols	Catechins, antocyanins, etc.
S-containing A.O.	Glutathione, lipoic acid, etc.

Antioxidants present in green leafy vegetables are numerous (Table 4) and must be incorporated in one way or another into the feed ration of the domesticated animal when that feed composition is returned to its wild standard in terms of fatty acid distribution. In fact, the presence or absence of essential nutrients in animal feeds is almost always additional (i.e., the presence of appropriately balanced amounts of essential omega-6/3 fatty acids in an animal feed also requires that appropriate amounts of antioxidant vitamins and minerals be present in that feed if only for stability reasons). On the other hand, modern feeds rich in saturated and omega-6 fatty acids require far fewer antioxidant vitamins and minerals.

8. FOOD TECHNOLOGY STANDARDS UNDER THE COLUMBUS CONCEPT

8.1. Land-Based-Animal-Derived Foods

Because alpha-linolenic acid (C18:3ω3; ALA) is metabolized faster than linoleic acid (C18:2ω6; LA) in land-based animals, wild-type animal feed (ω6:ω3-EFAs/TGs = 0.55:1 ± 0.05) transform into animal body fat balanced (ω6:ω3-EFAs/TGs = 1:1 ± 0.1) in essential fatty acids and doing so, into balanced sources of wild-type dietary lipids in humans (Table 5).

Table 5
Body Fat and Meat/Yolk in Standard and Wild-Type Animals

	ω6:ω3-EFAs/TGs		*%ω6/(ω6 + ω3)-HUFAs/TLs*		*Variations (Δ) in HUFAs*	
	Standard	*Wild-type*	*Standard*	*Wild-type*	*Δω6-HUFAs*	*Δω3-HUFAs*
Egg	>10:1	1:1 ± 0.1	60%	25%	–67.0%	+42.0%
Broiler	>10:1	1:1 ± 0.1	75%	25%	–36.0%	+175%
Pork	>10:1	1:1 ± 0.1	70%	25%(*)	–66.5%	±0%

Note: Table 5 presents experimental results obtained in commercially run standard and wild-type husbandries for layers, broilers, and pork. Standard and wild-type animals are distinguished from each other in their fat-deposit ω6:ω3-EFAs/TGs ratios and meat/yolk ω6/(ω6 + ω3)-HUFAs/TLs proportions (%); standard animals exhibit the characteristic >10:1 omega-6 to omega-3 EFAs ratio and >60% omega-6 HUFAs in total meat/yolk HUFAs, whereas their wild-type correspondents show a balanced 1:1 omega-6 to omega-3 EFAs ratio and ±25% omega-6 HUFAs in total tissue HUFAs, respectively. (*) In pork, the 25% is reached only if ALA is included in the HUFAs/TLs.

As seen in Table 5, the return to a balanced ω6:ω3-EFAs/TGs ratio in wild-type-animal body fat is associated with substantial changes in the content of highly unsaturated fatty acids (HUFAs) in their meat: omega-6 HUFAs (mainly AA) are substantially reduced (35–70%), whereas omega-3 HUFAs (EPA, DPA, DHA) increase in various proportions according to the species (from 0% in pork to 42% in eggs and 175% in broilers). Generally speaking, wild-type, animal-derived foods distinguish themselves from omega-3 rich and/or enriched foods. They are better described through their sharply reduced content of proinflammatory omega-6 HUFAs and their substantially improved content of anti-inflammatory omega-3 fatty acids and antioxidants, therefore contributing to healthily balanced blood-cholesterol levels (LDL-CC:HDL-CC) in humans. As demonstrated in Table 5, ω6/(ω6 + ω3)-HUFAs/TLs = 25% in animal meat is reached when their body fat comply with the Golden Game Standard (i.e., ω6:ω3-EFAs/TGs = 1:1).

8.2. Land-Based, Plant-Derived Foods

Wild-type, plant-derived foods are based on the same principle as that erected for wild-type, animal-derived foods (i.e., a balanced ratio of essential fatty acids [ω6:ω3-EFAs/TGs = 1:1] in the bulk triglyceride fraction of consumer products [e.g., table oil, bread, rice]). The reasoning behind this principle is that most fats from plant origins in the modern diet had no equivalent in the Paleolithic era and that these "new" fats in modern, energy-dense foods must then comply with the composition of the major source of fats in the hunter-gatherer diet (i.e., wild-animal body fat).

Modern table oils are extremely diverse in terms of their ω6:ω3-EFAs/TGs ratios, and the most commonly used in the modern diet (sunflower, peanut, and corn oils) rank high to very high (50 to 500) in the ω6:ω3-EFAs/TGs scale (Table 6). Mediterranean olive oil has an incidentally better pattern thanks to its high content of oleic acid (C18:1ω9) and resulting low content of linoleic acid (C18:2ω6), but it does not represent an ideal choice in terms of essential fatty acid content as such. On the other hand, Columbus Oil, a composite, naturally stabilized oil made of olive oil, flaxseed oil, and

Table 6
Table Oils Currently Available on the Market and Columbus Oil

		MUFA	*PUFA*			*Total*	
Lipid source	*SAFA*	*ω7 + ω9*	*ω6*	*ω3*	*ω6:ω3*	*P:S*	*S+M+P*
Sunflower	13	27	61	0.1	610	4.7	101.1
Peanut	14	43	35	0.1	350	2.5	92.1
Grapeseed	14	21	68	0.5	136	4.9	103.5
Corn	16	32	51	1	51	3.2	100
Palm	51	40	9	0.25	36	0.2	100.25
Olive (1)	16	70	13	0.6	22	0.8	100.6
Coconut	92	7	1.5	0.1	15	0.02	100.6
Olive (2)	15	79	5	0.6	8	0.4	99.6
Wheat germ	20	18	55	7	8	3.1	100
Soybean	16	22	54	7.5	7	3.8	99.5
Walnut	11	15	62	12	5	6.7	100
Canola	7	63	20	10	2	4.3	100
Columbus	**14**	**72**	**7**	**7**	**1**	**1.0**	**100**
Chia	9.7	6.7	19	64	0.3	8.6	99.4
Flax	6.9	19.5	15	57.5	0.26	10.5	98.9
Perilla	8.5	14.4	12.6	63.2	0.20	8.9	98.7

Adapted with permission from ref. *17*; *www.eatchia.com.*

green-type antioxidants, exhibits an ideal balanced fat to essential-fatty-acid ratio (P:M:S = 1:6:1 and ω6:ω3 = 1:1) for human daily consumption in partial or total substitution to animal fats.

Wheat bread and rice are two other food staples consumed in large quantities around the globe as a source of energy (carbohydrates). Their fat content is low (±1%), and their ω6:ω3-EFAs/TGs ratio is 10 to 20 times higher than ideal, making them excellent choices for rebalancing the average diet. A mixture of wheat-germ flour and rice with small amounts of flax, chia, and perilla seeds provides an easy model for designing land-based, wild-type, plant-derived food staples.

9. HEALTH PROMOTION AND DISEASE PREVENTION

When positioned on Lands' diagram, it becomes obvious that Columbus land-based, wild-type animal foods have similar patterns of blood HUFAs to humans suffering less from coronary heart disease (CHD); in contrast, modern animal foods share similar compositions to the blood of humans suffering the most from CHD (*see* Fig. 5).

Because wild-type foods simply fit Lands' diagram so nicely, determining dietary recommended intakes for essential fatty acids becomes an almost superfluous task. In fact, dietary essential fatty acids need just to be present in sufficient quantities and balanced to secure the 25% ratio of omega-6 HUFAs to total HUFAs in human blood and therefore maximum protection against heart diseases and probably against most modern degenerative diseases. How much of the green-type and game-type lipids

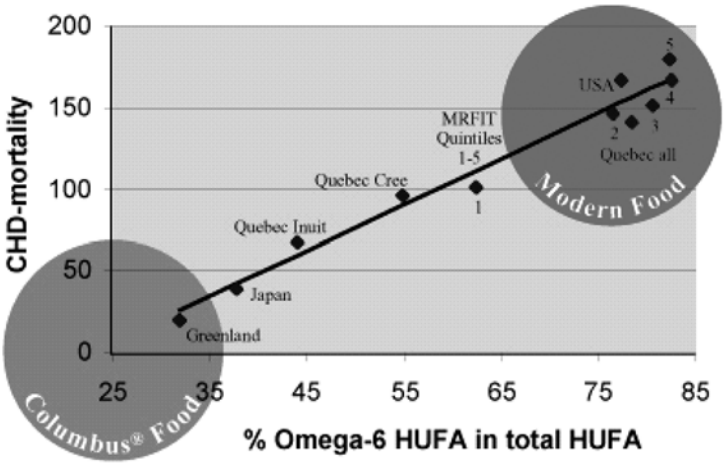

Fig. 5. Columbus and modern standard animal foods in lands' diagram. Shown is a Lands' diagram where %ω6/(ω6 + ω3)-HUFAs/TLs data from Table 5 for Columbus wild-type and Modern animal-derived foods are positioned according to the *x*-axis representing proportions of omega-6 HUFAs in total HUFAs in the different populations studied by Lands.

Table 7
Adequate Intakes for Essential and Conditionally-Essential Fatty Acids in Humans *(Vitamin-F)*

Fatty acid	*Infant (1)*	*Adult (2)*	*Pregnant (2)*
ω-6			
LA	600	4400	4400
AA	40	–	–
ω-3			
ALA	50	2200	2200
EPA	–	220	220
DHA	20	220	300

Note: Figures expressed in mg/2000-Cal diet, except for infants where numbers are given per kg body weight (BW) or per 150-mL (100-Cal) term infant formula. Adapted with permission from refs. *18* and *19*.

Abbr: LA: linoleic acid; ALA: alpha-linolenic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

should one eat every day to minimize the risk of heart disease is a question that was addressed in 1999 by an international expert committee, whereupon recommended daily intakes in close compliance with the wild-diet hypothesis were agreed upon and published (Table 7) *(19)*.

As seen, there seems to be an optimum ratio (5:1) of intake of green-type fatty acids (ALA: 2200 mg/d) and game-type fatty acids (EPA + DHA: 440 mg/d[1]) to be considered

[1]Based on an average 10%-conversion factor from ALA to EPA + DHA *(20)*, it is recommended to increase the daily intake of the latter to 660-mg when ALA is not sufficiently present in the diet and/or when the ω6:ω3 ratio of EFAs in the diet substantially deviates from unity.

Table 8
Nutrients and Non-nutrients, and Their Potential Influence on Diet/Health-Disease Relationships

	Nutrients		*Non-Nutrients*	
Type	*Essential nutrients*	*Nonessential nutrients*	*Functional components*	*Nonfunctional components*
Nature	EAAs, EFAs vitamins, minerals	AAs, MUFAs carbohydrates SFAs, cholesterol	non- or poorly-absorbed active food ingredients, physical, social and spiritual activities.	Residues, additives, contaminants stress, depression, sedentarism
Exchangeable	No	Yes	–	–
Contribution to DEI	±10%	±90%	Negligible	Negligible
Causal factors in diet/health disease relationships	Primary or independent	Secondary or nonindependent	Direct or indirect	Direct or indirect

Note: Essential nutrients are those that the body cannot synthesize or exchange; therefore, they must be found in the diet in appropriate amounts and proportions. When this is not the case, these nutrients become primary or independent risk factors in diet/health-disease relationships. Nonessential nutrients, on the other hand, represent the bulk of the diet and the diverse sources of energy. Cholesterol and saturated fats belong to this category. When ingested in excessive amounts, nonessential nutrients lead to obesity and its comorbidities and eventually show up as secondary or non-independent causal factors in diet/health-disease relationships. Non-nutrients can have positive/negative influences on regimen/health-disease relationships. They do not contribute substantially to the DEI.

Abbr: EAA: essential amino-acid; AA: non-essential amino-acid; EFA: essential fatty acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid.

as maximum protection against heart diseases. On the other hand, vegetarians and more specifically vegans should be recommended to balance their LA and ALA intakes to 4400 mg/d because they do not have direct access to the long-chain fatty acids EPA and DHA. Basically, the 1999 Expert Committee expresses the wild-diet hypothesis developed under the brand Columbus.

10. THE HIDDEN EVIDENCE

The wild-diet hypothesis does not include the former cholesterol-based diet-heart hypothesis or, in fact, it does, but from the more logical standpoint that cholesterol, just as saturated fatty acids, is not essential to humans and, therefore, cannot represent an independent risk factor in ill-health diseases (Table 8).

11. DIETARY CHOLESTEROL AND CVD

Ravnskov has written an entire book on "The Myth of Cholesterol" that shed some interesting light on how a scientific hypothesis has been transformed into public dogma, a story reminiscent to that experienced by Einstein with his Theory of Relativity.

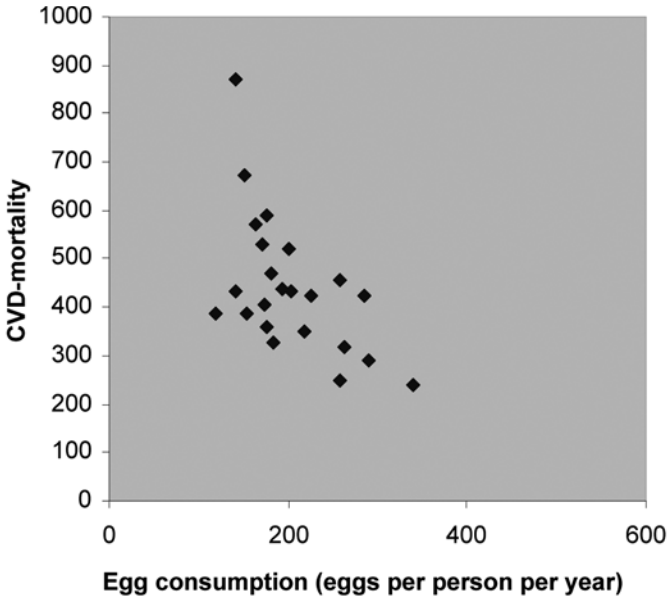

Fig. 6. CVD-mortality and egg consumption in 24 industrialized countries. The graph displays the mortality index in 24 industrialized countries, expressed as the frequency (#/100,000 inhabitants/yr) of fatal coronary outcomes (CVD-mortality) in the man-population of 35 to 74 yr of age as a function of the average annual egg consumption (eggs per person per year) in these countries.

However, and in contrast to Einstein's theory, the cholesterol-based, diet-heart hypothesis has not found confirmation, no matter how many human resources and financial means have been invested in clinical trials over the last 20 yr. At the same time, epidemiological data have provided ample information and confirmation that the hypothesis is wrong and have also suggested that reducing egg, milk, and meat consumption and blood cholesterol could be detrimental to human health.

12. DIETARY CHOLESTEROL AND CVD

Epidemiological data suggest that countries where egg consumption is the highest are those where the risk of fatal cardiovascular diseases is the lowest *(21)* (*see* Fig. 6).

It is interesting to note that the three countries (France, Mexico, Japan) where CVD-mortality and egg consumption are at the lower and higher ends, respectively, are those where omega-3 fatty acids (fish and greens) and antioxidant vitamins and minerals (vegetables, spices, wine) are omnipresent in the daily diet.

13. BLOOD CHOLESTEROL AND CHD

The former diet-heart relationship is strongly supported by the "7-country" study (*see* Fig. 7) *(22)*, which was once presented as the most solid epidemiological evidence that blood cholesterol was one of, if not the major culprit associated with CHD in humans. In fact, it appears from new insights in the published data based on Lands' observations (*see* Fig. 3) that the population absolute CHD mortality rate (ordinate) and the individual relative risk (slope) of CHD within each population differed substantially

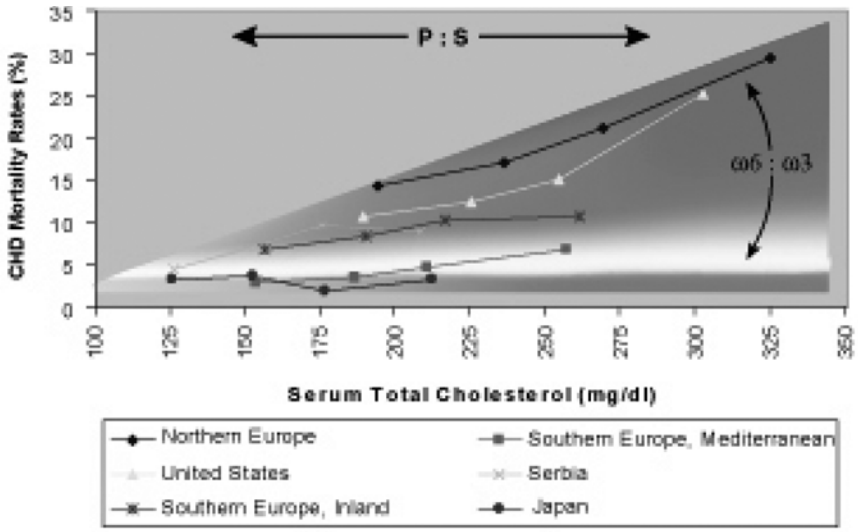

Fig. 7. The 7-country study. The graph displays the 25-yr coronary heart disease (CHD) mortality rates per baseline serum cholesterol quartile, adjusted for age, cigarette smoking, and systolic blood pressure, in 16 ethnic groups of 7 industrialized countries (5 European countries, the United States, and Japan). Observe that the large ordinate scale (0–35% CHD), reflecting the long timeframe (25 yr) of the study, reveals an impact of blood cholesterol not seen in the shorter timeframe clinical and epidemiological studies reported by Lands (*see* Fig. 4). Reprinted with permission from ref. *12*.

among cultures, and that those (Northern Europe, United States) suspected to be maintained at the time period of the study (1st part of 2nd half of the 20th century) on dietary patterns high (between 20:1 and 10:1) in ω6:ω3 ratios exhibited much higher absolute rates and relative risks of CHD than populations (Southern Europe, Mediterranean, Japan) that were supposed to naturally benefit from dietary patterns moderate or low (5 to 1:1) in ω6:ω3 ratios.

From this new angle, neither dietary saturated fats nor blood cholesterol appear as independent risk factors for CHD in the 25-yr follow-up study. Instead, the multiethnic absolute rates and relative risks of CHD seem to accurately respond to a single mathematical equation derived from Lands' diagram, such as:

$$\%\ \text{CHD-Mortality @ 25-yr} = 2.5 + (\text{TC} - 100) \times \text{TAN} \quad (\omega6/(\omega6+\omega3) - \text{HUFAs/TLs} - 0.25)$$

	Bood cholesterol (Vershueren)	×	Bood proportion of omega–6 HUFAs (Lands)
Risk factor	Secondary		Primary

The "7-Country Study" demonstrates how the peripheral tissue (or total blood lipids) %ω6/(ω6 + ω3)-HUFAs/TLs precipitates death by CHD as the only independent or

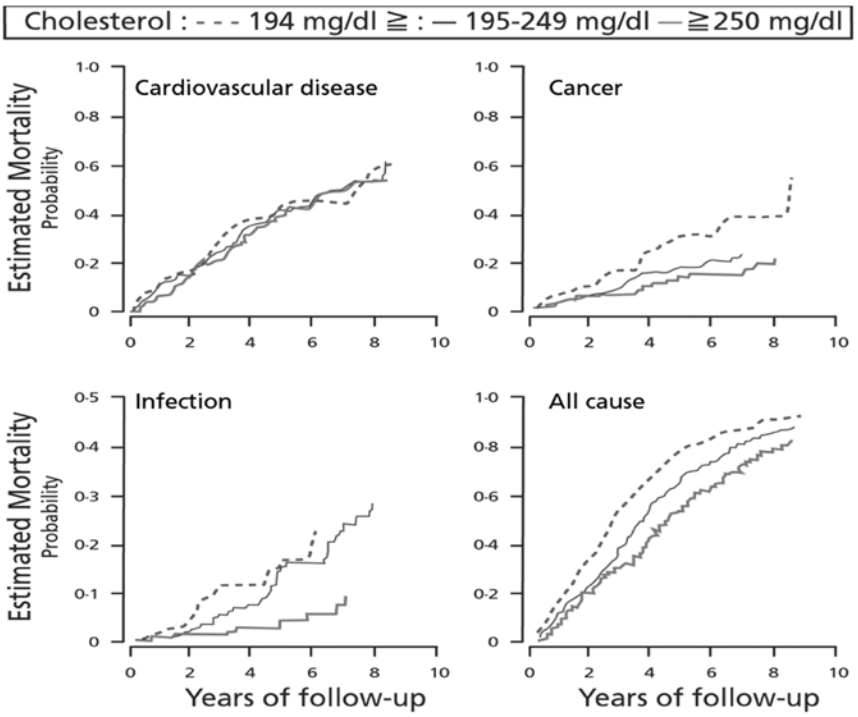

Fig. 8. Cholesterol levels and mortality rates in the elderly Oldest olds (over 85 yr old) followed for 10 yr in the Netherlands *(7)*. Among them, no significant correlation was found between serum-cholesterol level and CHD mortality. Instead, both cancer mortality and mortality from infectious diseases were higher when serum cholesterol was lower, and all causes of mortality were negatively correlated with serum-cholesterol levels. A similar conclusion was reached when people older then 70 yr were followed for 10 years in the USA *(8)*.

primary risk factor. This study, when reanalyzed from the essential fatty acid perspective, clearly shows that blood cholesterol is a non-independent secondary risk factor in the diet-heart hypothesis, because CHD is a low value constant (±2.5) at all TC values once blood ω6/(ω6 + ω3)-HUFAs/TLs is equal to 25%, whereas it seems to indeed worsen the body's response to challenges in essential fatty-acid-supplies, as perhaps also to other essential nutrients (trace elements, minerals, vitamins) when blood ω6/(ω6 + ω3)-HUFAs/TLs deviates from 25% or when dietary ω6:ω3 ratio deviates from 1:1, the Golden Game Standards in the Paleolithic diet.

14. BLOOD CHOLESTEROL AND LONGEVITY

It is generally observed that blood cholesterol increases with age for reasons that we still do not exactly know except that the response seems to be related to physiological changes associated with aging. In those who survive adulthood, epidemiological studies tend to conclude that a high blood-cholesterol level does promote longevity through improving body's defense against infections and cancers, with no influence whatsoever on risk of cardiovascular diseases, as expected from the wild-diet hypothesis (*see* Fig. 8).

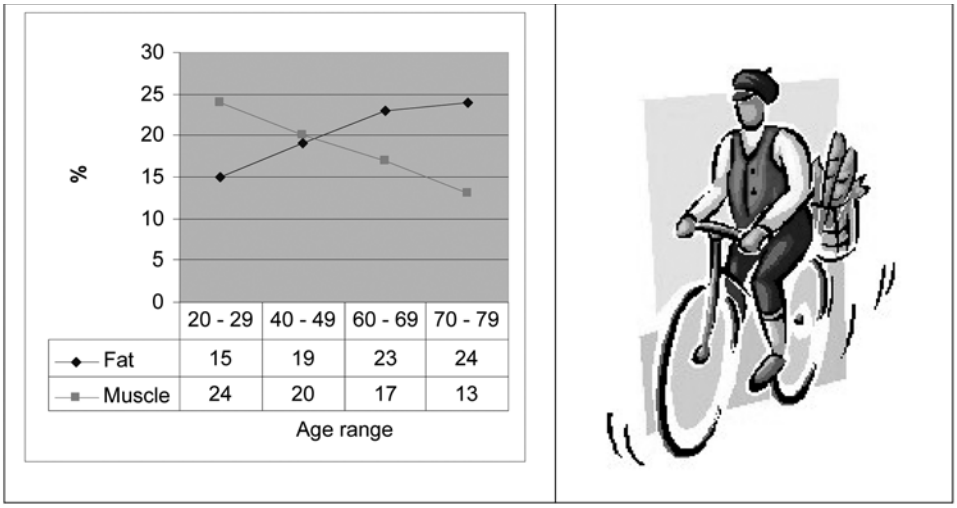

Fig. 9. Age-related changes in body composition in adult men. Normal or average values vary with age and sex. At all ages, women have a total body fat which, as a percentage of the body weight, is 10% greater than that of men. Thus, in a 25-yr-old man, fat is 15% of the body weight, whereas it is 25% in a woman of the same age.

Metabolic syndrome and fat/muscle redistribution are closely associated phenomena in the aging process. For some, unfortunately, the process starts at very early age. Given our origins as hunter-gatherers, retarding aging seems to have a lot to do with retarding fat/muscle redistribution (*see* Fig. 9).

In healthy men and women, fats overtake muscles with age *(23)*. At constant weight, this process witnesses progressive changes in lifestyle (i.e., reducing physical activity in association with reducing food intake). Because natural selection was substituted for human selection in mankind (read the opposite of *man selection in humankind,* as per past and still recent regrettable historical facts), the motor function of the body has lost close attention—to the benefit of brain function—leading to deviations in fat/muscle distribution in ever earlier ages. These physiological changes have been associated with increased blood-cholesterol and hormone levels, including steroids, insulin, and leptin.

There is no doubt that our body was genetically programmed to endure physical stress and that physical training is an important—though nonessential—factor of our health regimens. Just as with excess energy, inappropriate and/or lack of physical training can be seen as a non-independent secondary risk factor in the development of cardiovascular and other modern degenerative diseases. Therefore, maintaining a proper wild-type diet (read body fat/muscle composition) and physical activity (read body fat/muscle distribution) goes hand in hand with retarding age-related declining processes and co-morbidities. This conclusion was the central theme of debate at the 4th Congress on the Columbus® Concept, 12-15 October 2005, Beijing, China.

15. CONCLUSION

In nutritional sciences, there must be as many paradoxes as there are theories. Yet improved understanding supports the view that clearly identified, independent primary

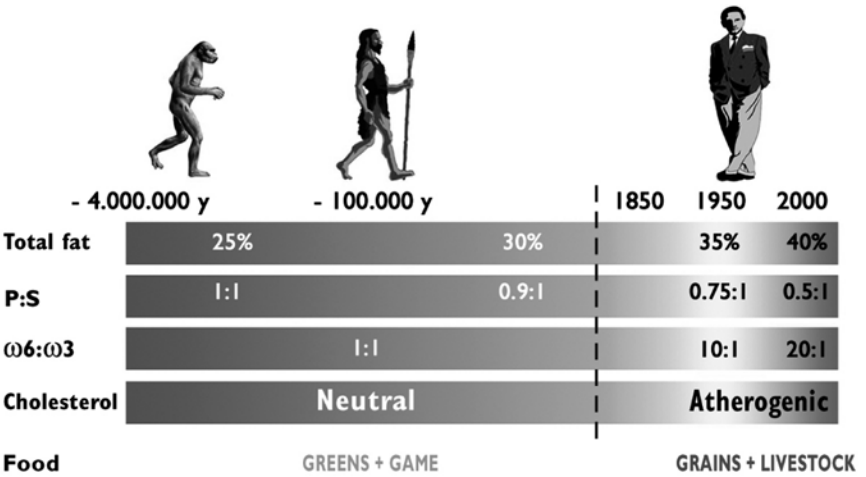

Fig. 10. Paleolithic diet and paleolithic blood cholesterol.

risk factors should first be isolated from nonindependent secondary ones (Table 7). Cholesterol and saturated fats are nonessential to the human body and therefore cannot as such represent independent primary risk factors to human-body dysfunctions. When associated with strong deviations in the food supply of essential nutrients—such as highly biased, essential-fatty-acid ratios and shortcuts in antioxidant vitamins and minerals—nonessential nutrients and their endogenous blood equivalents can affect the body's response to ordeals.

There is no doubt that the former 2D representation of "bad" and "good" cholesterols affecting health has not stood the test of time. The Columbus Hypothesis is that essential nutrients (essential fatty acids and antioxidant vitamins and minerals) are critical or primary risk factors for CHD in particular and modern degenerative diseases in general. Those critical factors are found in the Paleolithic diet because they have sustained human development. A striking difference in essential-nutrient distribution between modern and Paleolithic diets lies in the omega-6:omega-3 ratio of essential fats. Anthropological, epidemiological, clinical and scientific data converge towards an ideally balanced (1:1) dietary ratio. Modern animal- and plant-derived, wild-type Columbus Food can be created from modern, standard-food economic models. It is postulated that these foods will help rehabilitate dietary cholesterol and saturated fats for their potentially beneficial influence on blood cholesterol in the presence of appropriately balanced amounts of essential nutrients (*see* Fig. 10).

High blood cholesterol is inversely correlated with the prevalence of infections, cancers, and mental depression. Therefore, it is not only crucial but urgent that the Columbus wild-diet hypothesis be actively field tested.

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2 The Japanese Experience

High Cholesterol is Not an Important Risk Factor of All-Cause Mortality

Tomohito Hamazaki and Harumi Okuyama

Abstract

The risk from hypercholesterolemia has been overemphasized for years in Japan. Many of the Japanese epidemiological studies showed that high cholesterol levels, those between 240 and 260 mg/dL (6.2–6.7 mmol/L), are the safest in terms of all-cause mortality. Some epidemiological studies showed no upper limits of benefit for serum cholesterol levels. Therefore, treating subjects with 240 to 260 mg/dL of cholesterol references the paradox of treating those who are least likely to die. When serum cholesterol levels are estimated to be high, dietary intervention is often recommended. However, an observational study performed in Japan showed that cholesterol-reducing diets might increase the risk of acute myocardial infarction nearly three times. It is about time that we changed our attitude toward cholesterol especially in Japan.

Key Words: Cholesterol-lowering diet; guidelines; Japan Atherosclerosis Society; coronary heart disease; epidemiology.

1. INTRODUCTION

High blood cholesterol is a well-known risk factor for coronary heart disease (CHD). When all-cause mortality is considered, high-cholesterol levels may not be a sizable risk factor in Japan because the CHD mortality accounts only for 1/14 of all-cause deaths *(1)*.

Many of the Japanese lipid researchers believe that the lower the blood cholesterol levels are, the better *(2)*. If one needs to lower cholesterol levels, very often one tries to reduce daily cholesterol consumption *(3)*. Interestingly, there is no conclusive evidence for the beneficial effect of reducing cholesterol intake on the mortality from acute myocardial infarction (AMI) in Japan; let alone on all-cause death. The same is the case worldwide *(4)*.

This chapter explores why Japanese do not have to follow the guideline of the Japan Atherosclerosis Society *(2,5)*, and to lower blood cholesterol levels except for the extreme cases like familial hypercholesterolemia, which must be treated properly, or for secondary prevention cases.

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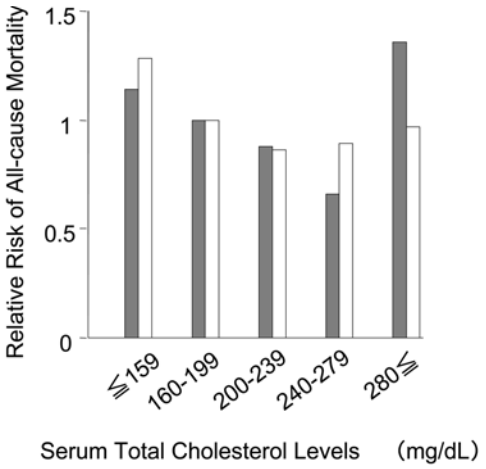

Fig. 1. Relationship between the total serum cholesterol levels and relative risk of all-cause mortality (Osaka). Residents of Osaka-hu were followed for 10.7 yr on average. Deaths in the first 2 yr were not counted. The group with160–199 mg/dL of total cholesterol was the referent group. Gray columns: men, open columns: women.

2. THE RELATIONSHIPS BETWEEN SERUM TOTAL CHOLESTEROL LEVELS AND ALL-CAUSE MORTALITY

Recently the relationships between total cholesterol levels and all-cause mortality became available from epidemiological studies with about 10,000 or more general citizens in Japan. Those relationships are shown in the following sections.

2.1. An Epidemiological Study From Osaka

Naito et al. *(6)* recruited 9662 residents (males = 35%) from 40 to 79 yr of age during routine health checkups performed in Osaka Prefecture between 1980 and 1995. Those subjects who had suffered from stroke and CHD were excluded from the study. Those subjects who died during the first 2 yr of observation after blood sampling were not included in the study in order to exclude seriously ill patients whose cholesterol levels might be very low. In males, cholesterol levels between 240 and 280 mg/dL (6.2–7.3 mmol/L) were the safest in terms of all-cause mortality, and in females cholesterol levels did not seem to matter except for those whose levels were below 160 mg/dL (4.1 mmol/L) (*see* Fig. 1).

2.2. Nippon Data

Nippon Data 80 is a well-designed large epidemiological study covering 300 areas from all over Japan *(7)*. Men and women (9216) not younger than 30 yr of age were recruited and followed for 13.2 yr from 1980. Hypercholesterolemia does not seem to be a risk factor of all-cause mortality after adjustment for age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking category and alcohol intake category (*see* Fig. 2).

2.3. An Epidemiological Study From Fukui Prefecture

The relationship between the serum total cholesterol and total death rate in Fukui citizens was more striking *(8)*. Fukui Health Center followed 26,249 subjects (males = 31%)

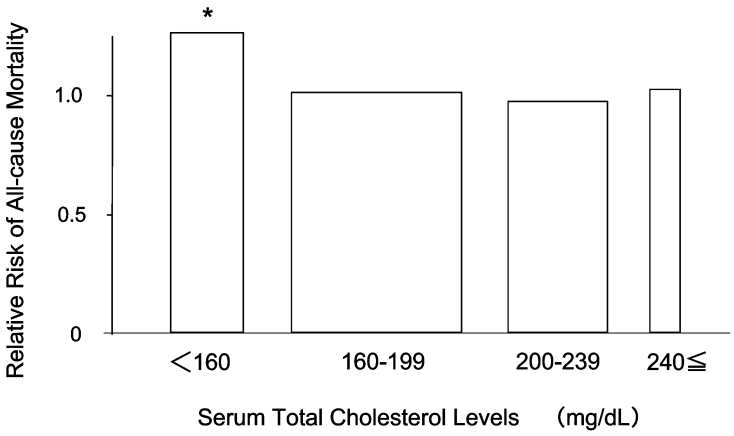

Fig. 2. Relationship between the total serum cholesterol levels and relative risk of all-cause mortality (Nippon Data). Relative risk of combined sexes was adjusted for age, serum albumin, body mass index, hypertension, diabetes, cigarette smoking category, and alcohol intake category. The trend did not change much even after deletion of the deaths during the first 5 yr. The width of the column is proportional to the number of subjects in the group. *Significantly different from the referent group (160–199 mg/dL).

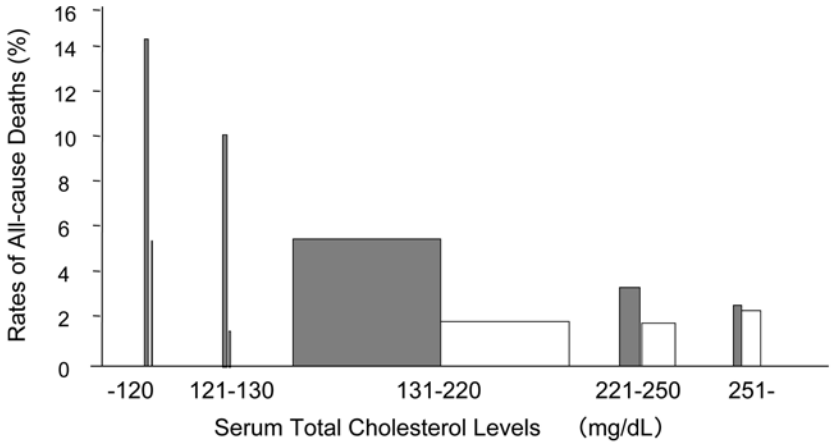

Fig. 3. Relationship between the total serum cholesterol levels and % death rates of all causes (Fukui). Residents of Fukui-city (26,249 subjects, males = 31%) were followed for 5 yr. All deaths were counted from the yr 1. The width of the column is proportional to the number of subjects in the group. P for trend was significant for men but not for women.

who were at least 40 yr old for 5 yr. There was no significant correlation between the cholesterol level and total death rate in women, but in men the higher the cholesterol levels were, the lower the total death rate (*see* Fig. 3). However, the deaths from cancer or other diseases during the first couple of years after blood sampling were not excluded, and unfortunately no such exclusion data were available.

2.4. Total Cholesterol and Death in Ibaragi-Prefecture

One of the largest epidemiological studies in Japan included 32,750 males and 63,959 female residents between 40 and 79 yr of age in Ibaragi-prefecture *(9)*.

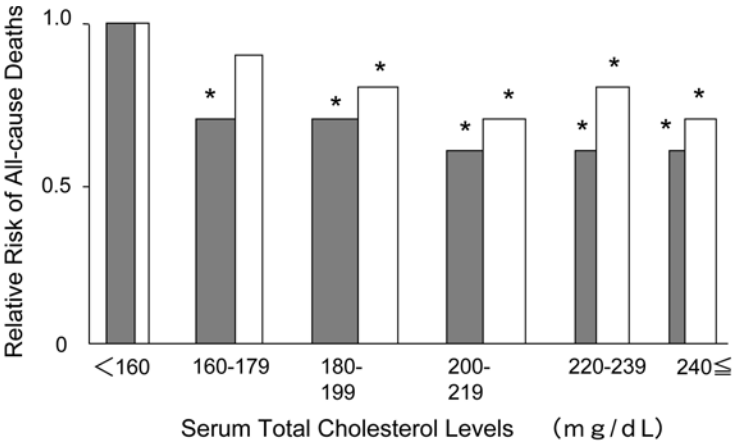

Fig. 4. Relationship between the total cholesterol levels and relative risk of all-cause mortality (Ibaragi). Residents of Ibaragi-prefecture (96,709, males = 34%) were followed for 5 yr and 2 mo on average. All deaths were counted from yr 1. The width of the column is proportional to the number of subjects in the group. *Significantly different from the referent group (<160 mg/dL).

Stroke patients were excluded in this study. During the follow-up period of 5 yr and 2 mo after blood sampling, a total of 2937 subjects died (1301 from cancer, 384 from stroke and 242 from CHD). There was no exclusion period of death-counting in this study. As shown in Fig. 4, the higher the total cholesterol levels were, the better the survival chance.

2.5. Another Epidemiological Study From Osaka

Tsuji et al. *(10)* took baseline data for residents of Moriguchi-city, Osaka, in 1997. After excluding subjects with cardiovascular disease at baseline, 16,461 subjects (average age: 54 ± 13 yr; males = 26%) were followed up for 5 yr. All-cause mortality is shown in Fig. 5. There was no exclusion period for death-counting. It appears that there were no upper limits for men, and that 280 mg/dL (7.3 mmol/L) might be the upper limits for women.

2.6. The Latest Report

Ogushi followed 9540 men and 18,942 women from 1999 to 2004 *(11)*. Their average ages were 66 ± 10 and 62.6 ± 10.8 yr, respectively. Those subjects were residents of Fukushima-prefecture and Kanagawa-prefecture. The relationship between the baseline serum total cholesterol and all-cause mortality during the follow-up period of 5 yr is shown in Fig. 6. The mortality of the subjects with serum cholesterol levels equal to or over 280 mg/dL (7.3 mmol/L) was very similar to that of the lowest mortality groups (levels between 220 and 239 mg/dL [5.7–6.2 mmol/L]).

3. SHOULD CHOLESTEROL LEVELS MORE THAN 240 MG/DL BE LOWERED AS JAPAN ATHEROSCLEROSIS SOCIETY RECOMMENDS?

Japan Atherosclerosis Society recommends that cholesterol levels of CHD-free subjects should be below 220 mg/dL (5.7 mmol/L) if there is any other risk factor; the

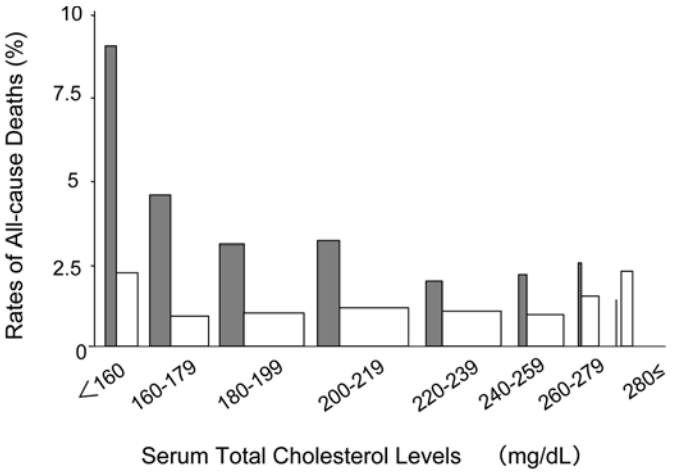

Fig. 5. Relationship between the total cholesterol levels and % death rates of all causes (Moriguchi). Residents of Moriguchi-city (16,461 subjects, average age: 54 ± 13 yr; males = 26%) were followed up for 5 yr. There was no exclusion period for death-rates counting. The width of the column is proportional to the number of subjects in the group.

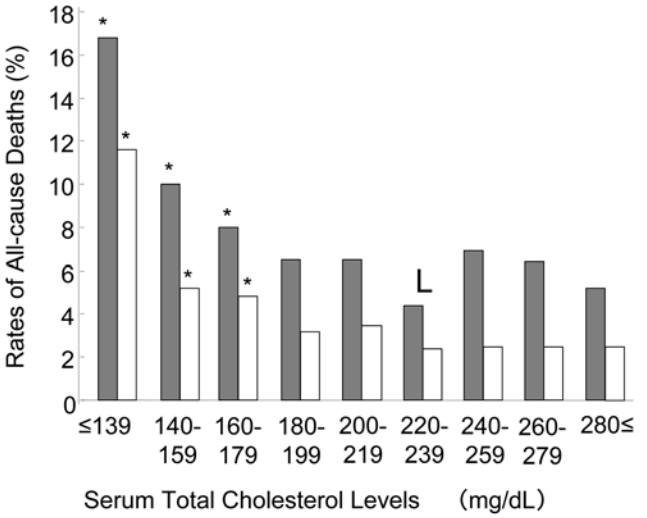

Fig. 6. Relationship between the total cholesterol levels and % death rates of all causes (Fukushima and Kanagawa). Residents of Fukushima- and Kanagawa-prefecture (9540 men and 18,942 women) were followed for 5 yr. All deaths were counted from the first year. *Significantly different from the referent group (220–239 mg/dL) indicated with L.

upper limits of serum cholesterol levels are set at 240 mg/dL (6.2 mmol/L) even if there is no risk factor *(5)*. This recommendation has a serious problem. As shown in Section 2, cholesterol levels around 250 mg/dL (6.5 mmol/L) are the best range for most of the studies in Japan in terms of all-cause mortality. Subjects belonging to the groups whose chances of all-cause deaths are the least must be the last people who need treatment.

Medical guidelines, in general, should disclose the background health information with regard to the parameters in question. Otherwise it would be difficult to make a

more balanced decision whether one should be treated or not. Unfortunately the guidelines from Japan Atherosclerosis Society did not describe any information about the relationship between total cholesterol levels and all-cause mortality in Japan *(2,5)*.

4. DO CHOLESTEROL-LOWERING DIETARY INTERVENTIONS BENEFIT HYPERCHOLESTEROLEMICS?

There are no large-scale prospective studies in Japan that describe the efficacy of cholesterol-lowering dietary intervention on AMI except for the following study.

4.1. Area-matched Control Study for Japan Lipid Intervention Trial

An interventional study using simvastatin (Japan Lipid Intervention Trial, J-LIT) was recently completed *(12)*. Because all the subjects in J-LIT were administered simvastatin and the results could not be compared with its own placebo group, another group of investigators *(13)* selected hyperlipidemic subjects comparable to those of J-LIT as controls (Area-matched Control Study for J-LIT). The recruited subjects were 4918 hypercholesterolemics (220–299 mg/dL; 5.7–7.8 mmol/L) without the history of AMI. They were regularly checked-up at 13 local health care centers located all over Japan. The recruited subjects were followed from 1993 to 1999. The proportion of subjects who received dietary instruction for hypercholesterolemia at the start of the study was 12.7 and 20.6% for those with blood cholesterol levels between 220 and 239 mg/dL (5.7–6.2 mmol/L) and 280 and 299 mg/dL (7.3–7.8 mmol/L), respectively. The subjects were followed under usual care and no intervention was made. During the follow-up period of 6 yr, 36 acute myocardial infarction cases were found *(13)*. Both a Cox hazard model (adjusted for age and sex) and a multivariate analysis including blood pressure and diet therapy showed that the risk of AMI was greater (2.30- and 2.89-fold, respectively) when dietary instruction was given to subjects at the start of the study. Diet education was the most serious risk of all as shown in Fig. 7. Why did that happen?

4.2. Cholesterol-reducing Diets Do Not Work

The principal instructions that were popular at that time can be summarized as follows: (i) reduce animal meat and increase plant products, (ii) replace butter with soft margarine, (iii) reduce foods containing much cholesterol like eggs, (iv) increase fiber intake, (v) increase soy and soy products, (vi) reduce calories if you are obese, (vii) stop smoking, and (viii) sometimes fatty fish was discouraged because of their high cholesterol contents.

The reason why dietary education was a serious risk factor in Area-matched Control Study for J-LIT was not clear from their study. However, the protocol of reducing animal fat and increasing plant oils made their diet high in linoleic acid. This type of diet may be harmful *(14)* or noneffective at best *(15)*. Margarine has trans-fatty acids. If subjects were recommended to eat less fatty fish, the results might be disastrous; in Japan those who eat fish 8 times/week has the least chance of CHD *(16)*.

Recently the Ministry of Health, Labour, and Welfare, in Japan essentially abolished the limits of cholesterol intakes, loosening the upper limits to 750 and 600 mg/d for men and women, respectively *(17)*. This decision is very wise and practical. We welcome that decision because very few people ingest cholesterol over these limits.

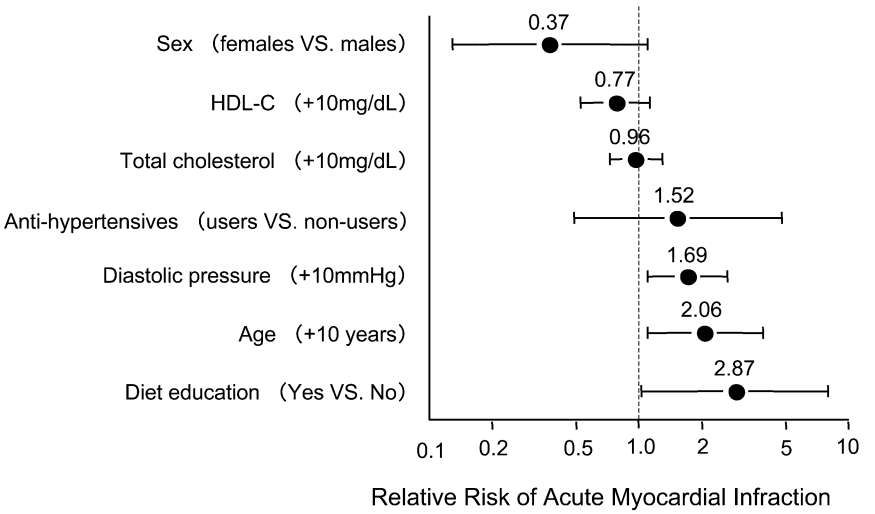

Fig. 7. Multivariate analysis of risk factors for acute myocardial infarction. After exclusion of cases with the history of AMI, 4918 hypercholesterolemics were followed for 6 yr. The risk of AMI was calculated. Data were adjusted for sex, age, blood pressure, and diet. Horizontal bars indicate 95% CI. Diet education at the start of the study was found to be the most serious risk.

5. CONCLUSIONS

Cholesterol levels up to 260 mg/dL (6.7 mmol/L) or 280 mg/dL (7.3 mmol/L) should not be regarded as a risk to be treated in Japanese people without CHD, because those high-cholesterol people have a low chance of dying earlier. Restrictions of dietary cholesterol intake may be of little value or even harmful.

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3 High ω6:ω3 Fatty Acid Ratio
The Israeli Experience

Gal Dubnov-Raz and Elliot M. Berry

Abstract

Consuming polyunsaturated fatty acids instead of saturated fat has been advocated for many years now, in an attempt to lower blood cholesterol and reduce atherosclerotic disease. In Israel, there is a high intake of ω6 fatty acids, a low total and trans fat intake, a high fruit and vegetable intake–but a similar prevalence of chronic diseases as in the rest of the western world. This *Israeli paradox* is probably due to the too-high intake of ω6 fatty acids, which can promote insulin resistance, increased atherogenesis and thrombosis, coronary events and cancer. Increasing ω3 fatty acid intake, by modifying fat content in foods and public education, seems a plausible way of raising the ω6:ω3 fatty acid ratio and lowering the rate of chronic diseases, in a population consuming a diet rich in ω6 fat.

Key Words: Omega-6; omega-3; polyunsaturated fatty acids; coronary artery disease; diet.

1. INTRODUCTION

The diet in the western world has changed considerably with industrialization. Many nutritional deficiencies seen in the past, or in the present in developing countries, are seldom encountered in western countries. Yet a "tip over the edge" has occurred, with overeating and obesity occurring widely. Consequently, a diet low in total calories, total fat, and saturated fat (substituted by the hypolipidemic ω6 polyunsaturated fatty acids [PUFA]), is a general recommendation to combat major health hazards: coronary artery disease (CAD), hypertension, obesity, and type-2 diabetes mellitus (DM). The dietary habits in Israel have always seemed to fit this recommendation, yet the rates of modern world diseases are either similar to those in the United States and Europe, or even higher. This occurs despite a lower rate of obesity, a lower average caloric intake, a higher consumption of fruit and vegetables and a higher intake of hypolipidemic ω6 PUFA and P/S ratio. A possible reason for this incongruity is "too much of a good thing": this high intake of ω6 fatty acids is possibly harmful *(1–5)*. The detrimental effects of ω6 fatty acids are mediated by the production of proinflammatory, thrombogenic, vasoactive, arrhythmogenic and carcinogenic eicosanoids, and include hyperinsulinemia and its associated metabolic disorders, atherosclerotic disease, cardiac arrhythmias, and cancer. In addition, this high ω6 intake may reduce the cardiac and vascular protective effects associated with ω3 PUFA *(6–18)*. Recently, there has been some debate as to whether ω3

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PUFA are indeed as cardioprotective and anti-arrhythmic as previously thought *(19–23)*, so additional data must be gathered. The Israeli diet allows us to demonstrate how several advantageous dietary characteristics can be overcome by a high ω6 intake and high ω6:ω3 ratio, thus preventing the expected health benefits.

2. THE ISRAELI PARADOX

The parent ω6 fatty acid is linoleic acid (LA, 18:2ω6). Studies utilizing adipose tissue analysis, which is a marker of chronic fatty acid ingestion, suggest that LA intake in Israel has increased during the past decades; the current estimate is between 10 and 12% of energy intake *(24,25)*. In the recent study by Kark et al. *(25)*, 90% of subjects consumed PUFA at more than 6% of energy, and a quarter consumed more than 12%. The P/S ratio was 0.9 on average, with a quarter of subjects reaching a value of 1.2 and above. A study from the 1980s showed that the LA content of subcutaneous fat in Israel ranged from 20 to 32%, with an average of 27% *(26)*. In a later study, the adipose tissue content of LA in Israel was still between 25 and 27%, twice than the 13% average of eight European countries in the EURAMIC study *(27)*. Thus, LA intake in Israel was very high along the years, as reflected by adipose tissue content. The self reported intake of total PUFA in Israel of the year 2000 is 8% of total energy *(28)*, but we assume this figure is slightly biased downwards.

There are several possible explanations as to why LA intake in Israel is so high. One is the high LA content of the marketed oils in the country, the most common being soy bean oil. It contains over 50% LA, and accounts for over 85% of vegetable oils in the market *(29)*. The second reason is kosher observance, held by 60% of households in Israel *(30)*; consequently, foods on the market are almost invariably kosher. In this diet, mixing of dairy and meat products is forbidden, so only vegetable-based oils and fats can be used in conjunction with meat. This markedly increases the use of vegetable oils and margarines, substituting for butter. Another feature of the kosher diet is that pork meat is forbidden, hence there is no use of lard. Again, vegetable oil is used instead. The third reason for high LA intake in Israel is nut consumption, as it is a common traditional snack. Yet these are not ω3-rich walnuts, but roasted nuts rich in ω6 (and sometimes monounsaturated fatty acids) such as pecan, almonds and pistachio. Though indiscriminative nut consumption was demonstrated to be cardioprotective *(9,10)*, we return to our claim that too much of a good thing (i.e., too much ω6 PUFA), results in untoward health effects.

In face of this high ω6 intake, we turn to ω3 PUFA consumption (both marine and plant sources), and the resultant ω6:ω3 ratio. In the United States it is around 10:1 *(31)*, in the UK about 16:1 *(32)*, in Europe about 10-14:1 *(24)*, and in Japan about 4:1 *(33)*. Estimated at between 22:1 and 26:1 in Israel, it is one of the highest in the world *(34)*, emanating from both a high ω6 intake, and a low ω3 intake. Fish are a major source of ω3 PUFA, yet the intake in Israel dropped from 17 kg/person/yr in 1950, to 10 kg/person/yr in 1973 *(29)*, to an estimated current 3.6 kg/person/yr (unpublished observations). For comparison, the average intake in industrialized countries is ~28 kg/person/yr *(35)*. The current self-reported intake of total ω3 fatty acids in Israel is estimated at 1.8gr/person/d *(28)*, suggesting a current ω6:ω3 ratio of 8.6:1. As mentioned, we believe that these figures are not accurate, but yet encouraging. It is possible that with continuing modernization and the global spread of large food chains, dietary habits are changing and becoming similar

throughout the western world. Because nutritional habits in the past are reflected in today's prevalence of chronic diseases, current dietary habits will be only reflected in the future. Similarly, the prevalence of chronic diseases that we see in Israel today can be a result of suboptimal nutrition in past years, just as "The fathers have eaten a sour grape, and the children's teeth are set on edge." (Jeremiah 31:29).

3. ADDITIONAL FEATURES OF THE ISRAELI DIET

The total amount of energy consumed in Israel during the late 1980s was lower than that of the United States and the European countries except Sweden *(36)*. The same was found for total fat intake, and all western countries consumed more total fat: about 120 gr/person/day in Israel, but 140 gr/person/day in UK, France and Italy, 150 gr/person/day in Canada, Spain and Greece, and 160 gr/person/day in the United States. In the early 1990s, data from the Food and Agricultural Organization of the United Nations (FAO) revealed that the caloric intake in Israel averaged 3115 kcal/person/d, compared with 3610 kcal/person/d in the USA, 3700 kcal/person/d in Belgium, and 3343 kcal/person/d in the Netherlands. Total fat intake was 114 gr/person/day, compared with much higher amounts in these countries, and was actually the lowest throughout the western world. Findings from the Lipid Research Clinics international program showed that in the subjects from Jerusalem, total energy intake, as well as total and saturated fat intake, were markedly lower than in the American subjects, while PUFA intake and the P:S ratio were higher *(37)*. In addition, butter intake in Israel in the mid 1990s was the lowest among 20 countries worldwide *(36)*. A high margarine intake naturally raises concern regarding trans fatty acid consumption. Yet trans fat intake in Israel of the 1980s was estimated at 6.5 gr/person/day, much lower than the estimated 9–12 gr/person/day in the United States *(26)*, thus suggesting another beneficial feature of the Israeli diet.

Regarding fruit and vegetable intake, data from the Israeli Ministry of Agriculture reveal a combined consumption of about 950 gr/person/day; this is higher than the FAO estimated average of between 600 and 800 gr/person/day in the United States and 300 and 400 in several European countries participating in the DAFNE III study. The Israeli MABAT study estimated and average of 4.5 fruit and vegetable servings/person/d in Israel *(28)*; in the United States, it was estimated at 3.37 in the same year of 2000 *(38)*.

Taken together, the Israeli diet, low in total energy and low in total, animal and trans fat, combined with a high intake PUFA, a high P:S ratio, and a high fruit and vegetable intake, seems a solid recipe for good health. Yet surprisingly, this does not translate into decreased morbidity.

4. CHRONIC DISEASES IN ISRAEL

Given the seemingly healthy dietary measures in Israel, a low prevalence of nutritionally-affected chronic diseases is anticipated. Yet the prevalence of CAD, diabetes mellitus and cancer in Israel is comparable with other western countries. Based on data from 1995 to 1997, CAD event rates were significantly higher among men and women from Jerusalem, compared to other Mediterranean and western countries participating in the MONICA project *(39)*. In Israel of 1995, the standardized mortality rate from CAD was 235 (males) and 168 (females) compared with 246 (males) and 131 (females) in the

United States and Europe *(40)*. The prevalence of diabetes mellitus in Israeli Jews in 1996 was 3.4%, *(40)* compared with 5.4% of United States citizens of the same year *(41)*, yet the rate of deaths from diabetes mellitus in Israel ranked first worldwide *(36)*. A high ω6:ω3 ratio is associated with insulin resistance *(42)*. The incidence of colorectal cancer was similar to that in United Kingdom, Canada, Italy, and United States whites *(36)*. Compared with the non-Jewish population of Israel, which have a more traditional diet, Jews have a much higher incidence of most types of cancer *(24,42)*. Therefore, the promising nutrition profile in Israel does not translate to less morbidity. This is termed the Israeli Paradox, where the high ω6 PUFA intake, which was supposed to translate into lower morbidity, actually counteracts the expected health benefits of the other favorable components of the Israeli diet.

5. FOR THE FUTURE

If the high ω6:ω3 ratio is indeed a harbinger of chronic illnesses, it can mathematically be lowered by either decreasing ω6 intake or increasing ω3 intake; biologically, it seems that both actions should be taken. Looking at the possible reasons for the high ω6 intake in Israel, the major one seems to be the high use of common vegetable oils, in part due to kosher diet restrictions. In addition, a low fish intake is found, probably the result of price restraints. Perhaps the most plausible way of decreasing such a high ω6:ω3 ratio would be at the manufacturer level-to substitute ω3 for ω6 in common market vegetable oils and additional foods. Such acts, with obvious additional costs, must be supported by either by government influence and funding, and by a major educational effort in terms of public health.

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4 Omega-6/Omega-3 Fatty Acid Ratio is Important for Health

Lessons From Genetically Modified Cells and Animals

Jing X. Kang

Abstract

An important nutritional question as to whether a balanced ω-6/ω-3 fatty acid ratio can reduce risk of modern diseases needs to be addressed in well-qualified experimental models. The recent development of genetic approaches to balancing ω-6 to ω-3 fatty acid ratio by expressing the *fat-1* gene (encoding an omega-3 fatty acid desaturase) in cultured mammalian cells and animals provides a new opportunity to address this issue. Using this technology, we have performed a series of experiments in both fat-1 transgenic cells (in vitro) and animals (in vivo). Our data obtained so far, as summarized in this chapter, support the notion that a reduced or balanced ratio of cellular ω-6 to ω-3 fatty acids is favorable for normal cell function and may reduce the risk of certain diseases, including cardiovascular disease, inflammatory disorders and cancer.

Key Words: Omega-6 fatty acids; omega-3 fatty acids; omega-6/omega-3 fatty acid ratio; fat-1 gene; omega-3 fatty acid desaturase; fatty acid composition; cardiovascular disease; inflammation; cancer.

1. INTRODUCTION

Omega-3 (ω-3) fatty acids are a special class of fats with the following characteristics: Structurally, they appear as a long hydrocarbon chain (18 or more carbons), containing three or more (up to six) double bonds. The first double bond occurs on the third carbon atom from the methyl end. These fatty acids are termed "essential" fatty acids because they cannot be produced by the body (animal or human) and must be supplied by the diet for good health. The source of ω-3 fatty acids is limited. Unlike other fatty acids, which are widely available in foodstuff, these ω-3 fatty acids are primarily found in fatty fish, certain vegetables and nuts. Functionally, ω-3 fatty acids can exert a wide range of effects on cell function. In addition to being a source of energy, these fatty acids can act as determinants of the physiochemical properties of cell membranes, as substrates for the production of signaling molecules or functioning mediators, and as modulators in the regulation of gene expression. Therefore, ω-3 fatty acids can profoundly affect the physiological activity and pathological process through different mechanisms.

The content of ω-3 fatty acids in the human diet underwent a dramatic change during evolution and civilization *(1)*. The foods available to our ancestors were quite different from what we eat today. It is believed that the "ancient" foods were rich in ω-3 fatty acids

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and had an equal balance of ω-6 to ω-3 fatty acids (i.e., ω-6/ω-3 ratio ≈ 1:1). Because such a fatty acid profile existed for so long during evolution, the human (animal) body established its genetic pattern based on that condition (i.e., loss or gain of certain fatty acid-related genes might have occurred to adapt to that dietary lipid profile). Under that dietary regime (rich in both ω-6 and ω-3 fatty acids), there was no need for genes that synthesize these fatty acids or convert one to another.

Today, however, the situation is quite different. Deviation began about 10,000 to 15,000 yr ago with the adoption of agriculture and animal husbandry, mainly ruminants. Modern agriculture with dietary dependence on grains led to an increase in total saturated fatty acids and the ω-6 polyunsaturated fatty acids linoleic and arachidonic acids. In the past century, the industrial revolution, the emergence of agribusiness with processed foods, grain-fattened livestock, and hydrogenation of vegetable fats have all further reduced the content of ω-3 fatty acids and increased ω-6 fatty acids. Consequently, the modern Western diet is deficient in ω-3 fatty acids and has too many of ω-6 fatty acids, resulting in a ω-6/ω-3 fatty acid ratio ranging from15:1 to 20:1 *(2)*. Obviously, this ratio is contradictory to our genetic profile established on a 1:1 ratio. Unfortunately, our body cannot adjust its gene profile in such a short period of time to adopt the new ratio.

The shift in the ω-6/ω-3 fatty acid ratio, especially the deficiency of ω-3 fatty acids, might have imposed a greater risk of modern diseases (e.g., cardiovascular disease, cancer) and thereby created a serious threat to public health *(1,2)*. This is evidenced by thousands of laboratory and human studies showing that deficiency of ω-3 fatty acids is associated with increased risk of several major diseases and that supplementation with ω-3 fatty acids exhibits beneficial effects on numerous clinical problems *(3,4)*. Among the many health benefits of ω-3 fatty acids, their cardioprotective, anti-inflammatory, anti-cancer, and neuroprotective effects have been most intensively investigated and are now becoming recognized. Notably, the most important effect of ω-3 fatty acids is prevention of sudden cardiac death, mainly through antiarrhythmic action *(5,6)*. Through a series of experiments ranging from the molecular level to human trials, our studies have documented the efficacy of the protective effect of ω-3 fatty acids against arrhythmias induced by various drugs/agents or by ischemia, and demonstrated that the protective actions result from modulation and stabilization of the electrical activity of the heart cells *(7,8)*. In light of the growing evidence for the cardioprotective effects of ω-3 fatty acids, the American Heart Association has recommended increased intake of fish for good heath and use of fish oil supplements for patents with documented coronary heart disease *(9)*.

The available sources of ω-3 fatty acids in our diets are marine vertebrates, stemming from the ability of single cell phytoplankton and algae to convert the parent ω-6 fatty acid, linoleic acid, to the parent ω-3 fatty acid, α-linolenic acid, which then enters the food chain and is further elongated and desaturated to produce the fish oil fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) *(1)*. As sources of edible fish in the oceans are being depleted by over fishing and the market price of fish keeps rising, no one knows where ω-3 fatty acids will come from to meet future demand.

In short, the reality we are facing is: that (i) the demand for ω-3 fatty acids is growing because of their great health benefits, but the source is limited and (ii) ω-6 fatty acids are highly abundant in our food and too prevalent in our body, but we are unable to convert ω-6 to ω-3 fatty acids because humans, as well as most animals, do not have the

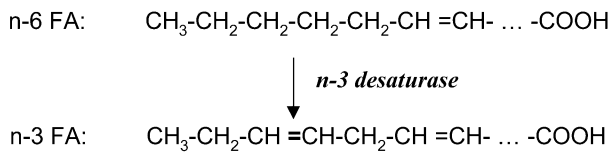

Fig. 1. Conversion of ω-6 fatty acids (FA) to ω-3 fatty acids by an ω-3 desaturase that does not exist in mammalian cells. The ω-3 desaturase can catalyze introduction of a double bond into ω-6 fatty acids at the ω-3 position of their hydrocarbon chains to form ω-3 fatty acids.

required gene. So, what can we do about it? The current practice is to enrich tissues with ω-3 fatty acids and balance the ω-6/ω-3 ratio by feeding animals exogenous ω-3 fatty acids (e.g., fish meal or other marine products); this is unsustainable.

These problems prompted us to wonder whether biotechnology could be used to enable mammalian cells and animals to produce ω-3 fatty acids from the ω-6 type. Because some lower life forms, such as plants, microorganisms, and *Caenorhabditis elegans*, are able to convert ω-6 to ω-3 fatty acids, and some of the responsible genes have been cloned *(10)*, our idea was to transfer a converting enzyme gene from these species to mammals. The gene we used was *fat-1* from the roundworm *C. elegans*. It encodes a ω-3 fatty acid desaturase that introduces a double bond into ω-6 fatty acids at the ω-3 position of their hydrocarbon chains to form ω-3 fatty acids (*see* Fig. 1). We tested our idea first in cultured cells, followed by generations of transgenic animals.

2. TRANSGENIC CELLS (IN VITRO STUDIES)

In order to introduce the *fat-1* gene into mammalian cells efficiently, we used a virus-mediated gene transfer strategy. We constructed a recombinant adenovirus (Ad.GFP.*fat-1*) carrying both the *fat-1* gene and the green fluorescent protein (GFP) gene and another adenovirus (Ad.GFP) carrying the GFP gene alone (as a control) and used them to infect various mammalian cells, including heart cells, neurons, endothelial cells and human cancer cell lines *(11–15)*.

Following the virus-mediated gene transfer, cellular lipids were extracted and fatty acid composition was analyzed by gas chromatography to determine whether the expression of the *fat-1* gene in mammalian cells changes their lipid profile. Our results showed that the fatty acid profiles were remarkably different between cells expressing the *fat-1* gene and control cells *(11–15)*. In cells expressing the *fat-1* gene (ω-3 fatty acid desaturase), all types of ω-6 fatty acids were largely converted to corresponding ω-3 fatty acids, namely, 18:2n-6 to 18:3n-3, 20:2n-6 to 20:3n-3, 20:3n-6 to 20:4n-3, 20:4n-6 to 20:5n-3, 22:4n-6 to 22:5n-3 and 22:5n-6 to 22:6n-3 (*see* Fig. 2). As a result, the amount of ω-3 fatty acids in the fat-1 transgenic cells significantly increased, whereas the levels of ω-6 fatty acids decreased. This led to a dramatic reduction in the ω-6/ω-3 ratio, from between 9:1 and 15:1 in the control cells to about 1:1 in the fat-1 cells (Table 1) *(11)*. Similar effects were observed in all cell types that we have tested *(12–15)*. To examine whether the gene transfer-induced alteration in the ratio of ω-6 to ω-3 can lead to a change in the profile of eicosanoids generated by the cells, we measured the production of prostaglandin E_2 (PGE_2), one of the major eicosanoids derived from 20:4n-6 (AA), in the fat-1 and control cells by using an enzyme immunoassay *(11–14)*. We found that the amount of prostaglandin E_2 produced by the

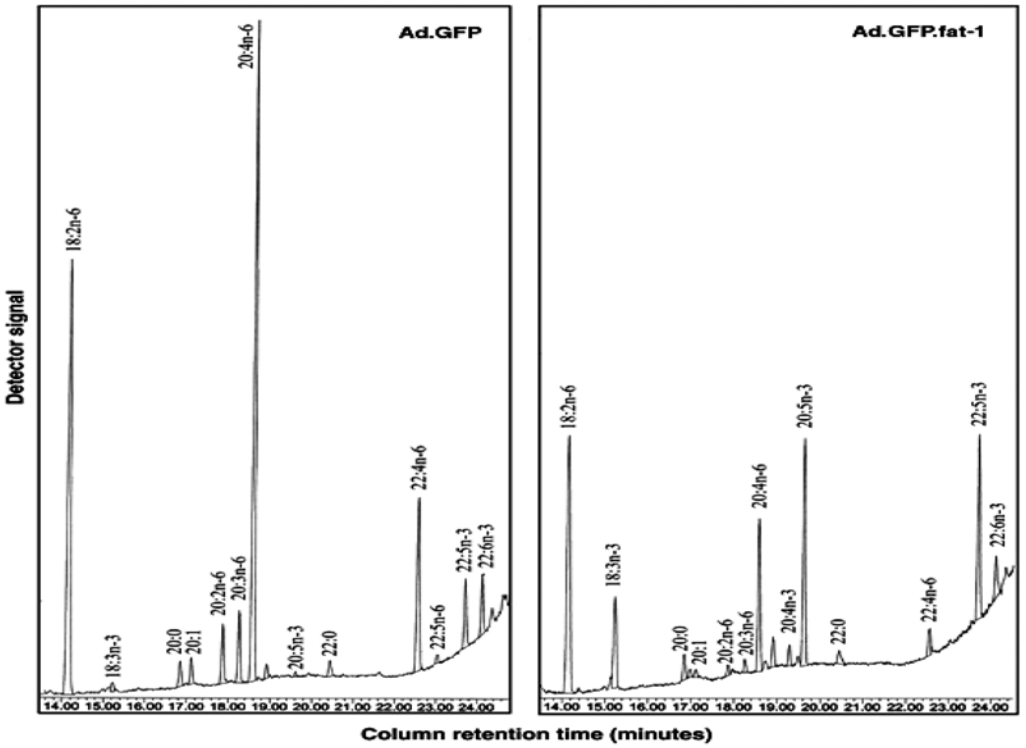

Fig. 2. Partial gas chromatograph traces showing fatty acid profiles of total cellular lipids extracted from cultured neonatal rat cardiac myocytes infected with Ad.GFP (control) and the myocytes infected with Ad.GFP.*fat-1*.

cells expressing the *fat-1* gene was significantly lower than that produced by the control cells (30–50% reduction) *(11–13)*. Apparently, this genetic approach is highly effective in balancing the cellular ω-6/ω-3 fatty acid ratio and in modifying the generation of eicosanoids.

In comparison with supplementation, the gene transfer technology is superior because it not only enhances absolute quantity of ω-3 fatty acids but also simultaneously decreases the level of ω-6 fatty acids. Unlike supplementation with exogenous fatty acids, this genetic approach needs no incorporation of exogenous fatty acids into cells to alter ω-6/ω-3 ratio and therefore does not change the total amount of cellular fatty acids (i.e., no difference in lipid mass between treated cells and control cells). Thus, the transgenic cells created by this technology can serve as a unique model for elucidating the significance of the ratio of ω-6 to ω-3 fatty acids.

Next, we determined whether the gene transfer-induced change in the ω-6/ω-3 ratio would provide the same beneficial effects as observed with fatty acid supplementation. Our previous studies have demonstrated an antiarrhythmic effect of ω-3 fatty acids when added to cardiac myocytes *(7)*. To see whether the gene transfer can provide a similar protective effect, neonatal rat cardiac myocytes expressing the *fat-1* gene were tested for their susceptibility to arrhythmias induced by arrhythmogenic agents, such as high concentrations of extracellular calcium. As shown in Fig. 3, when challenged with

Table 1
Polyunsaturated Fatty Acid Composition of Total Cellular Lipids From the Control Heart Cells and the Transgenic Cells Expressing a *C. elegans fat-1* cDNA

Mol % of total fatty acids	*Control*	*Fat-1*
n-8 Polyunsaturated		
18:2n-6	14.2[a]	8.2[b]
20:2n-6	1.2[a]	0.3[b]
20:3n-6	1.6[a]	0.4[b]
20:4n-6	15.2[a]	4.1[b]
22:4n-6	4.4[a]	1.0[b]
22:5n-6	0.2[a]	0.0[b]
Total	36.8[a]	15.0[b]
n-3 Polyunsaturated		
18:3n-3	0.2[a]	3.6[b]
20:4n-3	0.0[a]	0.6[b]
20:5n-3	0.1[a]	6.1[b]
20:5n-3	1.2[a]	5.8[b]
22:6n-3	1.0[a]	1.3[b]
Total	2.5[a]	17.4[b]
n-6/n-3 Ratio	14.7[a]	0.8[b]

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly ($p < 0.01$) between control and fat-1.

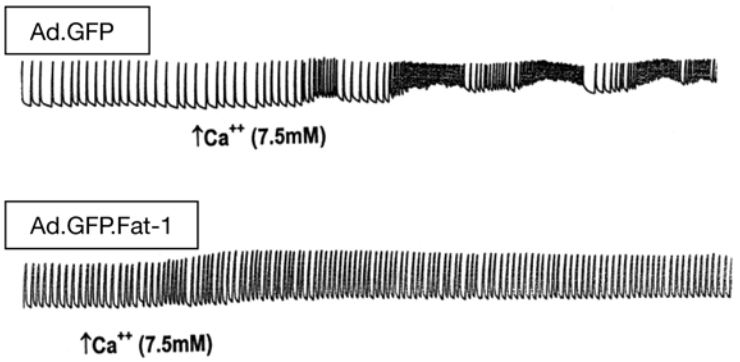

Fig. 3. Effect of expression of the *C. elegans* ω-3 fatty acid desaturase in cardiac myocytes on their susceptibility to arrhythmia. Cultured (spontaneously beating) neonatal rat cardiac myocytes infected with Ad.GFP (control) or Ad.GFP.*fat-1* were challenged with 7.5 m*M* extracellular calcium. The control cells promptly exhibited arrhythmia (spasmodic contractures and fibrillation), whereas the fat-1 cells could sustain regular beating.

a high [Ca^{2+}] (7.5 m*M*), the control cells promptly exhibited arrhythmia characterized by spasmodic contractures and fibrillation, whereas the cells expressing the *fat-1* gene sustained regular beating (resistant to the arrhythmogenic stimulus), similar to the effect of ω-3 fatty acid supplementation *(7)*. This suggests that gene transfer of the ω-3 desaturase into heart cells can provide the antiarrhythmic effect of ω-3 fatty acids.

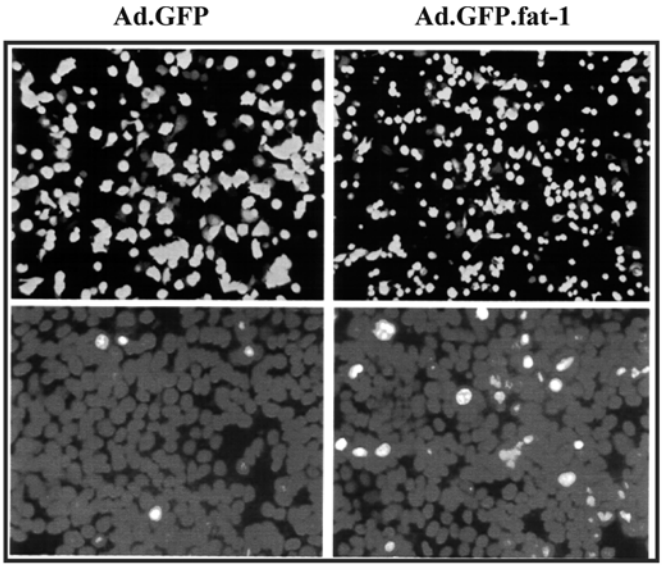

Fig. 4. The gene transfer induces apoptosis of MCF-7 cells. MCF-7 cells were infected with Ad.GFP (*left:* control) or Ad.GFP.*fat-1* (*right*). Three days after infection, cell death was examined using a fluorescence microscope. *Upper panels*: infected cells were directly visualized at 510 nm of blue light. *Lower panels*: Cells were stained with Hoechst dye for nuclei and observed under 480 nm fluorescent light. The brighter blue spots are the nuclei of apoptotic cells.

A number of human cancer cell lines have been tested for their responses to the expression of the *fat-1* gene. In human breast cancer cells (MCF-7), gene transfer of the ω-3 desaturase resulted in reduction of both cellular ω-6/ω-3 fatty acid ratio from 12.0 to 0.8 and the level of PGE_2 by about 40%, leading to an increase in apoptotic cell death and a decrease in cell proliferation *(13)*. As shown in Fig. 4, a large number of the cells expressing the *fat-1* gene underwent apoptosis, as indicated by morphological changes (small and round, or fragmentation) and nuclear staining (bright spots). Statistical analysis of apoptotic cell counts showed that between 30 and 50% of cells infected with Ad.GFP.*fat-1* were apoptotic, whereas only 10% dead cells were found in the control group (infected with Ad.GFP). Cell proliferation assays indicated that proliferative activity of cells infected with Ad.GFP.*fat-1* was significantly lower than that of cells infected with Ad.GFP. Accordingly, the total number of viable cells in the cells infected with Ad.GFP.*fat-1* was about 30% less than that in the control cells. In addition, DNA microarray assays showed that the gene transfer-induced change in the ω-6/ω-3 fatty acid ratio could result in a down-regulation of a number of genes involved in cell proliferation, adhesion, angiogenesis and invasion, and an up-regulation of apoptosis-inducing genes in MDA-MB-231 cells (unpublished data). In human lung cancer A549 cells *(15)*, reduction of the cellular ω-6/ω-3 fatty acid ratio from 5 to 1 as a result of the expression of *fat-1* cDNA led to cell growth arrest, and, more importantly, reduced invasive potential as evidenced by a decrease in cell adhesion, migration and expression of invasion-related genes (*see* Fig. 5). These results are consistent with the reported anti-cancer effects of ω-3 fatty acid supplementation *(16–18)*.

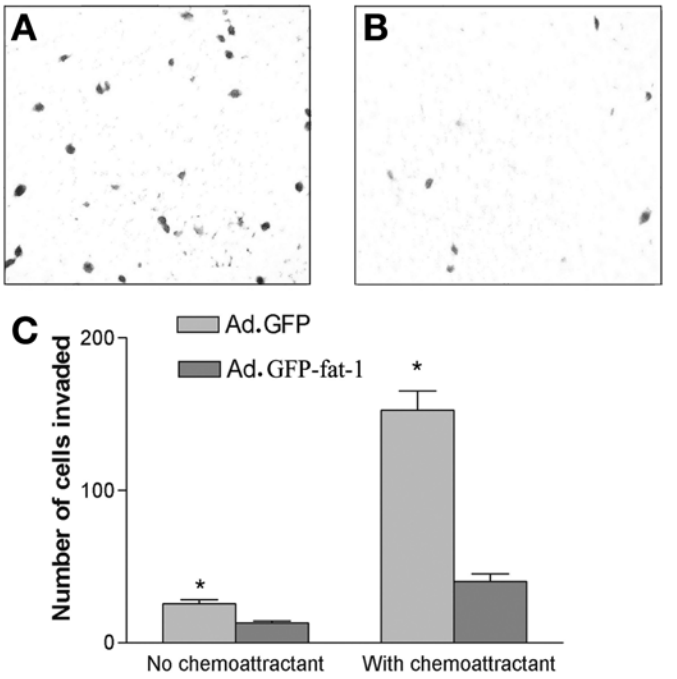

Fig. 5. *Fat-1* expression decreased the invasive potential of A549 cells. The cells that invaded through the matrigel-coated *trans*-well inserts toward chemoattractant were stained with 1% crystal purple. Photographs were taken at a magnification of ×100. **(A)** Control cells infected with Ad.GFP; **(B)** transgenic cells infected with Ad.GFP-*fat-1*; and **(C)** the cells invading through the matrigel were counted under microscope in five predetermined fields at ×100. Each sample was assayed in triplicate.

In primary culture of human umbilical vein endothelial cells (HUVEC), expression of *fat-1* significantly reduced the ω-6/ω-3 fatty acid ratio from about 9 to 1 *(14)*. This change in the cellular ω-6/ω-3 ratio led to a decrease in the surface expression of adhesion molecules (markers of inflammation). The quantity of the adhesion molecules (as determined by immunoassay), E-Selectin, ICAM-1, and VCAM-1 was reduced by 42, 43, and 57%, respectively, in response to cytokine exposure (tumor necrosis factor [TNF]-α 5 u/mL, 4 h) *(14)*. We then examined whether changes in the adhesion molecule profile were sufficient to alter endothelial interactions with monocytes, the most prevalent white blood cell type found in atherosclerotic lesions. Under laminar flow and a defined shear stress of ~2 dynes/cm^2, *fat-1* compared to control vector infected HUVEC supported about 50% less firm adhesion with almost no effect on the rolling interactions of THP-1 cells *(14)*. These results indicate that expression of the *fat-1* gene in HUVEC inhibits cytokine induction of the endothelial inflammatory response and firm adhesion of monocytes, suggesting that a balanced ω-6/ω-3 fatty acid ratio may have an antiatherosclerotic effect.

We have also determined the effect of *fat-1* expression on neuronal apoptosis.We found that the expression of the *fat-1* gene, which could significantly reduce the neuronal ω-6/ω-3 fatty acid ratio from 6 to 1.5 and the production of prostaglandin E_2 by 20%, resulted in protection from growth factor-withdrawal-induced apoptotic cell death of rat cortical neurons *(12)*. Following gene transfer, apoptosis was induced by 24 h of growth

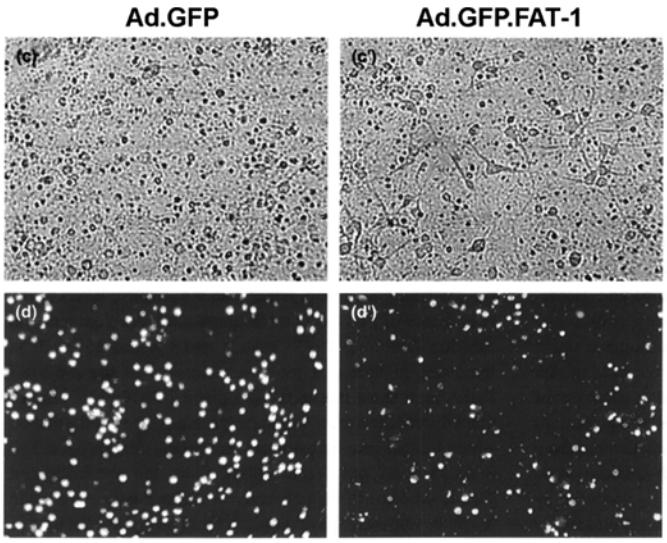

Fig. 6. Photomicrographs showing the protective effect of *fat-1* gene on neuronal apoptosis. Cells were infected with Ad-GFP (left panels, control) or Ad-GFP-*fat-1* (right panels). Cell death was examined after 24 h of growth factor withdrawal using a fluorescent microscope. Bright-field (*upper panels*) and Hoescht staining (*lower panels)* images showing appoptotic cells.

factor withdrawal and detected by Hoechst staining. As shown in Fig. 6, cortical cultures infected with the Ad.GFP*.fat-1* underwent about 60% less apoptosis than those infected with Ad.GFP *(12)*. Accordingly, cell viability assays indicate that the viability of Ad.GFP*.fat-1* cells was significantly (~50%) higher than that of cells infected with Ad.GFP. These observations confirm the protective effects of ω-3 fatty acid supplementation on neuron death *(19,20)* and highlight the importance of the ω-6/ω-3 ratio in this neuroprotective effect.

These in vitro studies clearly indicate that expression of the *C. elegans fat-1* gene (n-3 fatty acid desaturase) in mammalian cells can quickly and dramatically balance the ω-6/ω-3 fatty acid ratio, alter the eicosanoid profile, and consequently provide the beneficial effects of ω-3 fatty acids without the need for supplementation with exogenous ω-3 fatty acids.

3. TRANSGENIC ANIMALS (IN VIVO STUDIES)

On the basis of the in vitro results, we proceeded with generation of fat-1 transgenic animals capable of producing ω-3 from ω-6 fatty acids.

To heterologously express the *C. elegans* ω-3 fatty acid desaturase in mice, we modified the *fat-1* gene by optimizing the codon usage for mammalian cells and coupled it to a chicken β-actin promoter. We then microinjected the expression vector into fertilized eggs to produce transgenic mouse lines.We have now successfully generated mice expressing the *fat-1* gene *(21)*.

Both transgenic and wild type mice are maintained on a diet high in ω-6 fatty acids (mainly linoleic acid) with very little ω-3 fatty acids (~0.1% of total fat supplied). Under this dietary regime, wild-type mice have little or no ω-3 fatty acid in their

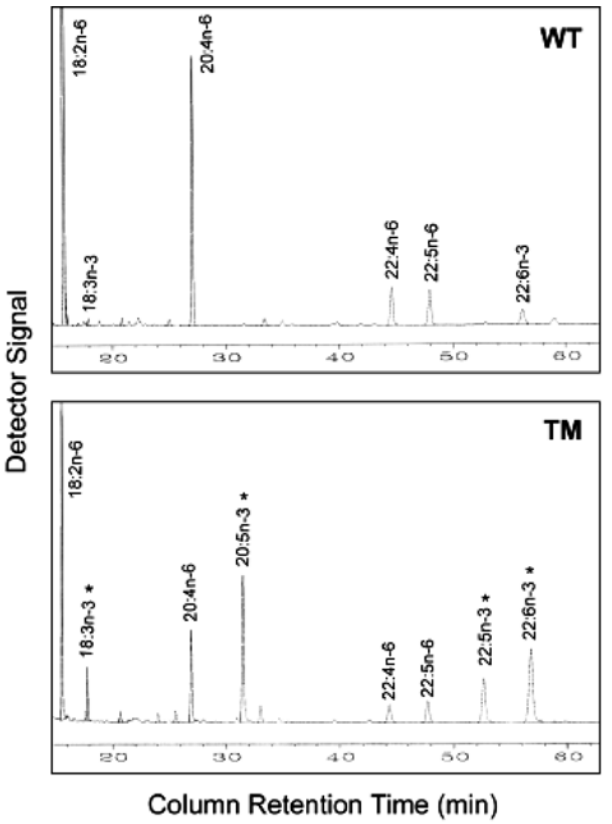

Fig. 7. Partial gas chromatograph traces showing the polyunsaturated fatty acid profiles of total lipids extracted from skeletal muscles of a wild-type mouse (WT) (*upper panel*) and a fat-1 transgenic mouse (TM) (*lower panel*). Both the wild-type and transgenic mice were 8-wk-old females and fed with the same diet. Note, the levels of ω-6 polyunsaturated acids (18:2n-6, 20:4n-6, 22:4n-6, and 22:5n-6) are remarkably lower whereas ω-3 fatty acids (marked with *) are abundant in the transgenic muscle (*lower panel*) compared with the wild-type muscle in which there is very little ω-3 fatty acid (*upper panel*).

tissues because the animals naturally cannot produce ω-3 from ω-6 fatty acids, whereas the *fat-1* transgenic mice have significant amounts of ω-3 fatty acids (derived from ω-6 fatty acids) in their tissues *(21)*. Figure 7 shows the differential fatty acid profiles of total lipids extracted from skeletal muscles of age and sex-matched wild type and transgenic mice. In the wild type animals, the polyunsaturated fatty acids found in the tissues are mainly (98%) the ω-6 linoleic acid (LA, 18:n-6) and arachidonic acid (AA, 20:4n-6) with trace (or undetectable) amounts of ω-3 fatty acids. In contrast, there are large amounts of ω-3 polyunsaturated fatty acids, including linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) in the tissues of the transgenic mice. Accordingly, the levels of the ω-6 fatty acids LA and AA in the transgenic tissues are significantly reduced, indicating a conversion of ω-6 to ω-3 fatty acids. The resulting ratio of ω-6 to ω-3 fatty acids in the tissues of transgenic animals is close to 1. This ω-3 rich profile of lipid with a balanced ratio of ω-6 to ω-3 and an even more balanced

Table 2
Comparison of the ω-6/ω-3 Ratios and AA/(EPA+DPA+DHA) Ratio in Various Organs and Tissues Between a Wild-Type Mouse (WT) and a fat-1 Transgenic Mouse (TG)*

	*ω-6/ω-3***		*AA/(EPA + DPA + DHA)*	
	WT	*TG*	*WT*	*TG*
Muscle	49.0	0.7	11.3	0.4
Milk	32.7	5.7	15.7	2.5
RBC	46.6	2.9	27.0	1.6
Heart	22.8	1.8	14.3	0.9
Brain	3.9	0.8	3.6	0.7
Liver	26.0	2.5	12.5	0.9
Kidney	16.5	1.7	11.9	1.2
Lung	32.3	2.2	19.8	1.2
Spleen	23.8	2.4	17.3	1.5

Notes: *Both the wild-type and transgenic mice were 8-wk-old females and fed with the same diet.

**The ω-6/ω-3 fatty acid ratio is (18:2n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6)/(18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3).

AA/(EPA+DPA+DHA) can be observed in all of the organs/tissues, including muscle and milk (Table 2). Our data clearly show that the transgenic mice expressing the *fat-1* gene are capable of producing ω-3 fatty acids from ω-6 fatty acids, resulting in enrichment of ω-3 fatty acids in their organs/tissues without the need for dietary ω-3s, which is impossible in wild type mammals.

The transgenic mice appear to be normal and healthy. To date, several (more than five) generations of transgenic mouse lines have been examined and their tissue fatty acid profiles show consistently high levels of ω-3 fatty acids, indicating the transgene is transmittable.

With this model, one can readily address specific effects of ω-3 fatty acids or the ω-6/ω-3 ratio in any organs/tissues, ranging from gene expression to physiological activity during the entire life cycle. For studies comparing the effects of ω-3 and ω-6 fatty acids or various ω-6/ω-3 ratios, the use of this model has advantages: (i) It can eliminate the lengthy and costly feeding of different special diets for comparative studies (ω-6 vs ω-3) and (ii) It can provide more reliable and definitive results than dietary manipulation. For experiments designed to examine the effects of two different ratios of ω-6/ω-3 fatty acids, two different diets must conventionally be fed to the animals to establish different fatty acid profiles. Feeding two different diets for months makes it impossible to keep everything identical between two groups of animals. Many variables can arise from the diets and the feeding procedures, including the concentration, impurity, or unwanted components of the oils used (e.g., fish oil vs corn oil), flavor, sensitivity to oxidation, diet storage, and duration of diet change, which can confound the absolute content of ω-3 fatty acids and the ω-6/ω-3 fatty acid ratio. These potential confounding factors in the diet can lead to unreliable (inconsistent or conflicting)

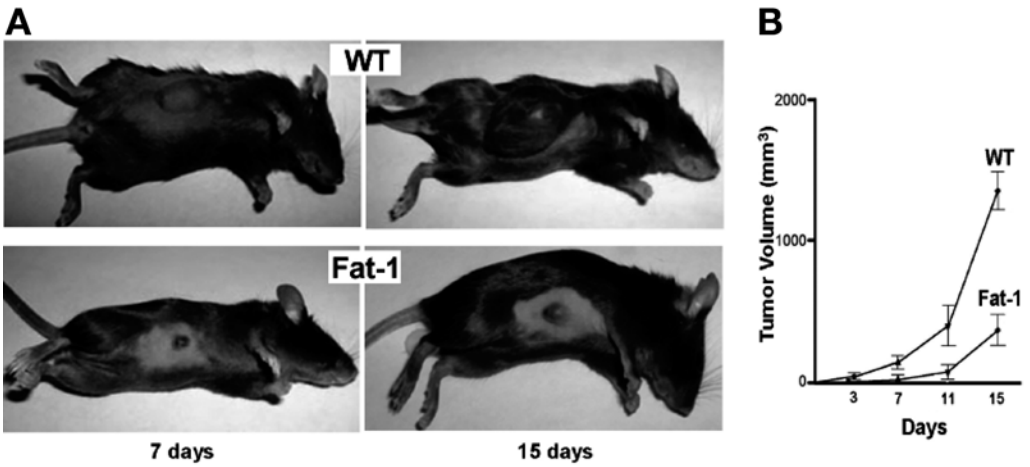

Fig. 8. (A) Different sizes of melanomas in wild-type and fat-1 transgenic (FAT-1) mice at two different time points. A number of 5×10^6 viable cells in 50 µL of PBS were injected subcutaneously into each of 10 transgenic and 10 wild-type littermates (2-mo-old female). On days 7 and 15 after cell implantation, animals were anesthetized briefly with isofluorane, and the tumor was examined and photographed using a digital camera. **(B)** Growth rates of melanomas in wild-type and transgenic mice. Tumor growth was monitored at the indicated time points by measuring the length (*L*) and width (*w*) of the tumor with a caliper and calculating tumor volume on the basis of the following formula: volume = $(1/2)Lw^2$. The points are mean values ±SD of 10 tumors ($n = 10$) for the wild-type (WT) group or of 7 tumors ($n = 7$) for fat-1 transgenic group (FAT-1).

results. Availability of fat-1 transgenic mice allows us to produce two different fatty acid profiles in experimental animals by feeding them a single identical diet so that carefully-controlled studies can be performed without the interference of the potential confounding factors of diet; and (iii) fat-1 transgenic mouse lines can be genetically crossed with established disease models (transgenic or knockout animals) to generate combined (*fat-1* plus disease gene) models, which can address the effects of n-3 fatty acids and/or n-6/n-3 ratio on the pathogenesis and therapy of the disease. Therefore, fat-1 transgenic mice will serve as a new tool for ω-3 fatty acid research.

Recently, we have begun to explore the potential differences in physiology and pathophysiology between the transgenic and wild type mice, and have obtained some exciting preliminary data. For example, we implanted mouse melanoma B16 cells into the transgenic and wild-type mice (maintained on a high ω-6/ω-3 diet) and examined the incidence of tumor formation and tumor growth rate *(22)*. The results showed a dramatic inhibition of melanoma formation and growth in fat-1 transgenic mice. Over an observation period of 15 d, all ($n = 10$) wild-type mice developed palpable tumors by day 3, whereas only 7 out of 10 transgenic mice developed minor tumors palpable by day 7 or even day 10. The tumor growth rate (mean tumor volume over time) was much slower in the fat-1 transgenic mice when compared to the wild-type mice. The smallest tumor in the wild type group was still bigger than the biggest one in the transgenic group (Fig. 8) *(22)*.

There is also a marked difference in both PGE_2 and PGE_3 contents between the fat-1 and wild-type mice. In wild type mice, both the tumor and surrounding tissues have a large amount of PGE_2, but little PGE_3. In contrast, the amounts of PGE_2 in the tumor

and surrounding tissues of fat-1 transgenic mice are lower than those of wild type mice, whereas PGE_3 are highly abundant (more than that of its counterpart PGE_2) in both the tumor and stromal tissues of fat-1 transgenic mice *(22)*. In addition, we found that PTEN (phosphatase and tensin homolog deleted on chromosome 10), a critical tumor suppressor in melanoma tumorigenesis, was dramatically up-regulated (16- to 32-fold increase) in the melanoma tumors of fat-1 transgenic mice *(22)*. These data suggest an anti-cancer (anti-melanoma) effect of n-3 fatty acids through, at least in part, activation of PTEN pathway mediated by PGE_3 *(22)*.

In a DSS-induced colitis model, fat-1 mice had a much lighter inflammatory response than wild-type animals *(23)*. Induction of colitis typically results in significant changes in body weight, stool consistency, appearance of fecal blood and general status, all associated with human and experimental DSS-colitis *(24)*. Fat-1 mice showed significantly less body weight loss and a delayed progression of diarrhea, but no apparent change in fecal bleeding. Interestingly, fat-1 transgenic mice showed a recovery beginning from the second day after the stop of DSS-exposure, whereas wild-type mice exhibited a continued loss of body weight during the 3 d after cessation of DSS. These clinical manifestations were reflected in the macroscopic pathological changes. Multiple adhesions, strictures, and a massive thickening of the colon were observed in wild-type mice, but not in fat-1 animals. Furthermore, colon shortening amounted to 35% in wild-type mice, but only 15% in fat-1 transgenic mice when compared with that of untreated control mice. Microscopic assessment of the distal part of the colon revealed that severity and thickness of the inflammatory infiltrate as well as the extent of epithelial damage were significantly alleviated in fat-1 mice. All hallmarks of colitis were reduced in fat-1 mice, save minor punctate erosions and a few ulcerations. In contrast, wild-type mice showed a massive fibrinous exudate on the luminal surface and marked epithelial infiltrate of leukocytes, as well as severe submucosal edema and diffuse ulcerations of the mucosa. These findings indicate that fat-1 transgenic mice, rich in ω-3 fatty acids, are protected from inflammation (*see* Fig. 9) *(23)*.

We next examined whether the protection from colitis observed in fat-1 transgenics had an impact on inflammation-related gene expression. TNF-α plays a critical role in inflammatory bowel disease (IBD) and its overexpression is associated with an IBD-like phenotype in mice *(25)*. Concordant with the protective action of the increased ω-3 fatty acid status was a decrease in NFκB protein activity, as determined by activation of p65 protein, as well as in TNF-α. In addition, other prominent inflammatory markers, such as iNOS and IL-1β *(26)*, were dampened in the transgenic fat-1 mice *(23)*.

In addition, we observed that the mRNA levels of intestinal trefoil factor 3 (TFF3), a factor important in maintenance and repair of the intestinal mucosa *(27)*, was increased in the colons of fat-1 mice. The intercellular tight junction protein Zonula occludens 1 (ZO-1), which is important in epithelial integrity *(28)*, was also sustained in fat-1 transgenic animals. Furthermore, expression of Toll interacting protein (TOLLIP), a downstream inhibitor of the TLR pathway that mediates inflammatory response *(29)*, was higher in fat-1 transgenic mice. These results suggest an enhanced mucosal defence status in the fat-1 mice *(23)*.

When lungs were challenged with LPS or bleomycin, pulmonary inflammation and fibrosis in the transgenic mice seemed to be less severe than that in wild-type animals; the fat-1 transgenic mice have lower levels of blood triglyceride and higher HDL; in

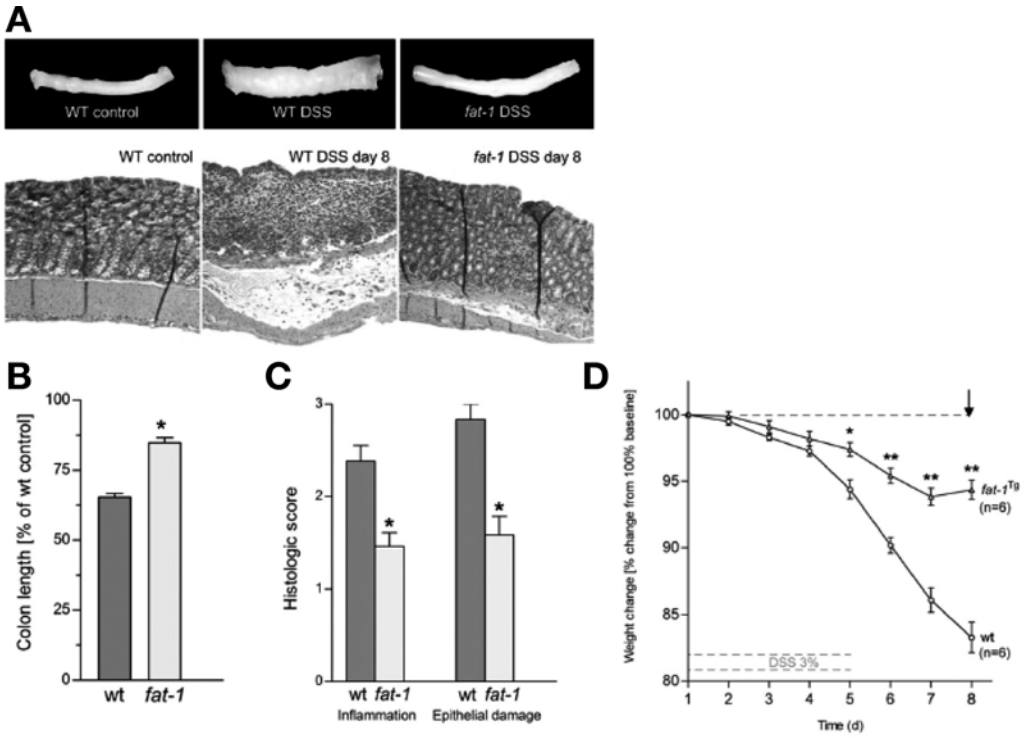

Fig. 9. Colon inflammation activity in wild type and fat-1 transgenic mice. **(A)** Macroscopic view (*upper panel*), microscopic Hematoxylin & Eosin staining (*lower panel*) of the distal colon in WT control (*left*), DSS-treated WT non-transgenic littermates (*center*) and fat-1 (*right*) mice. **(B)** Colon shortening as hallmark of DSS induced colonic damage, is reduced in fat-1 mice. *, $p < 0.01$ vs WT DSS-treated animals. **(C)** Histopathological scores for colonic inflammatory infiltration and epithelial damage in WT and fat-1 mice. *, $p < 0.01$ vs WT DSS. **(D)** Body weight change from 100% baseline over 8 d in fat-1 mice and WT littermates ($n = 6$ for each group), 5 d DSS treatment and 3 d normal drinking water. *, $p < 0.05$ vs WT DSS; **, $p < 0.01$ versus WT DSS. Mice were sacrificed on day 8 (*arrow*) and samples taken for further analysis.

addition, the wild-type mice behaviorally exhibit hyperactivity, whereas the fat-1 mice did not (unpublished data). More interestingly, we recently generated *ob/ob* plus *fat-1* and *ApoE*$^{-/-}$ plus *fat-1* mouse lines and examined the development of metabolic syndrome and atherosclerosis in these animals. Our preliminary data showed some beneficial effects of ω-3 fatty acids on these conditions (unpublished data). Our observations in vivo obtained so far are consistent with the in vitro data and support the notion that a balanced ratio of ω-6/ω-3 fatty acids is more desirable in reducing the risk of many diseases.

Following the successful generation of fat-1 transgenic mice, we moved to create larger transgenic animals: livestock. Very recently, in collaboration with Dr. Yifan Dai (University of Pittsburgh) and Dr. Randall S. Prather (University of Missouri-Columbia), we successfully created fat-1 transgenic pigs. The pork from the fat-1 animals is rich in ω-3 fatty acids and has an ω-6/ω-3 ratio of close to 1 *(30)*. This supports the feasibility of using our genetic approach to provide land-based sources of ω-3 fatty acids. Generation of other fat-1 transgenic livestock (chicken, cows, and fish) is underway in our laboratory. Once these food products become available in the market, we could thus

achieve a ω-6/ω-3 ratio approximating 1.0 without the public having to make stringent changes in their diets. In other words, if a person does not like or can't get fish, he would simply eat his favorite hamburger, hotdog, or eggs to obtain the required amount of healthy ω-3 fatty acids. Thus, our discovery provides not only a new tool for ω-3 fatty acid research, but also a new strategy for producing ω-3 fatty acid-rich foodstuff (e.g., meat, milk, and eggs) by generating large fat-1 transgenic animals/livestock. This genetic approach might be a cost-effective and sustainable way to produce ω-3 essential fatty acids for the increasing demand in the future.

4. CONCLUSION

The genetic approach to modifying ω-6 to ω-3 fatty acid ratio by expressing the ω-3 fatty acid desaturase (*fat-1* gene) in cultured mammalian cells and animals provide a new opportunity to investigate the biological importance of these fatty acids. Our data derived from the fat-1 transgenic cells and animals support the notion that a reduced or balanced ratio of cellular ω-6 to ω-3 fatty acids is favorable for normal cell function and may reduce the risk of certain diseases, such as cardiovascular disease, inflammatory disorders and cancer. More studies using this model to address the effects of ω-3 fatty acids are warranted.

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5 Tissue Omega-6 and Omega-3 Fatty Acids in Health and Disease

William E. Lands

Abstract

The proportions of n-3 and n-6 highly unsaturated fatty acids (HUFA) in tissue HUFA are useful biomarkers that characterize an individual's average daily dietary intake of essential fatty acids, predict the likely intensity of n-6 eicosanoid actions during tissue responses and estimate the likely risk of death from coronary heart disease. The proportions vary predictably from 20 to 80% depending on the voluntary food choices of each individual. To help individuals identify and select specific food choices that meet each person's preferences in taste and maintain their tissue proportions of n-3 and n-6 HUFA at a personally desired level of risk aversion, an interactive menu planning program with an interactive United States Department of Agriculture (USDA) nutrient database of nearly 12,000 food servings is downloadable free from *http://efaeducation.nih.gov/sig/kim.html*.

Key Words: Arrhythmia; biomarkers; eicosanoids; essential fatty acids (EFA); highly unsaturated fatty acids (HUFA); inflammation; omega-3; omega-6; thrombosis.

1. ω-6 AND ω-3 BIOMARKERS

Essential fatty acids (EFA) have three important actions: forming specific lipoprotein structures necessary in specialized tissues; forming complexes that regulate gene expression; and forming highly active autacoids that act on tissue receptors. The third action is the focus of this chapter. Interpreting how the balance between n-3 and n-6 EFA in diets and tissues can give both good and bad actions requires careful quantitative evaluation of the abundance and actions of their derived 20- and 22-carbon highly unsaturated fatty acids (HUFA). The evaluation is greatly aided by having a fast, economical and reliable biomarker for tissue HUFA status to relate to its dietary inputs and its likely clinical outcome. Discussions of methodology at the 2004 ISSFAL meeting in Brighton, UK noted that high-throughput robotic gas chromatographic assays of 50 microliter samples of whole blood might handle 200 samples/d, 1400 samples/wk, and 70,000 samples/yr at laboratory costs near $5/sample. The new technology improves an earlier rapid analysis *(1)* and allows economical monitoring of large-scale nutrition interventions designed for primary prevention of chronic diseases.

The proportions of n-3 and n-6 HUFA in tissue HUFA are useful biomarkers that characterize an individual's average daily dietary intake of EFA, predict the likely intensity

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of n-6 eicosanoid actions during tissue responses and estimate the likely risk of death from coronary heart disease (CHD). The proportions vary predictably from 20 to 80% *(2,3)* (*seehttp://efaeducation.nih.gov/sig/hufacalc.html*) depending on the voluntary food choices of each individual. Tissue triglyceride contents of the weight percent (wt%) of 18-carbon polyunsaturated EFA, 18:2n-6 and 18:3n-3, have a simple linear relationship to their dietary abundance *(2)* expressed as percent of daily calories (en%). However, some confusion in the field comes from inappropriate attempts to treat curvilinear diet-tissue relationships with linear associations. Clinical interpretations of chronic disorders that have excessive n-6 eicosanoid actions require recognizing that dietary en% abundances of 18:2n-6 and 18:3n-3 are related hyperbolically to the proportions of their HUFA derivatives in tissues, to their biological efficacy as EFA, and to their effect on n-6 eicosanoid response intensity.

The very sensitive hyperbolic response of tissue HUFA composition and physiological growth gives a strong linear increase with very small amounts (<0.5 en%) of dietary 18:2n-6 and 18:3n-3 *(4,5)* (*see: http://efaeducation.nih.gov/sig/dri.html*), whereas those measures seem to be unresponsive to more than 2 en% in diets. In the United States, where the quintiles of dietary intake of linoleate have mean values of 2.9, 4.4, 5.5, 6.9, and 10.3 en% *(6)*, many researchers seem misled by the apparent metabolic unresponsiveness at these high intakes. Such high EFA intakes also suppress the formation of n-9 HUFA, making the percent of n-3 in HUFA equal to 100 minus the percent of n-6 in HUFA. The almost undetectable amounts of 20:3n-9 in human HUFA may be a biomarker of EFA intakes being far above what is necessary.

An empirical hyperbolic equation was developed *(2)* (*see http://efaeducation.nih.gov/sig/hufacalc.html*) to predict quantitatively the diet-tissue relationship for experimental animals and humans. Similar selectivities of the lipid metabolizing enzymes in rodents and humans makes the data from laboratory animals *(7)* useful in predicting results with humans. In addition, a given dietary regimen produces similar proportions of n-6 in the HUFA of plasma, erythrocytes, and liver *(7)* (as well as in heart, leukocytes, and kidney). As a result, monitoring the proportion of n-6 in the HUFA of blood is useful when estimating the likely HUFA proportions in other tissues. Stark et al. *(8)* showed similar values for human plasma and erythrocytes, confirming that future biomarker monitoring can be done with whole blood samples for greater speed and convenience.

Two easily prevented imbalances in food intake (imbalanced energy intake to expenditure and imbalanced n-6 to n-3 EFA) are initial causes of much of the chronic inflammation, thrombosis and arrhythmia *(9)* (*http://efaeducation.nih.gov/sig/chainofevents.ppt*) that leads to disease and death. Two biomarkers (the mg/dL of blood cholesterol and the %n-6 in HUFA) help monitor the impact of those two dietary imbalances, respectively. The first biomarker results from high influx of food energy to the liver, and it is a metabolic end-product of isoprenoid biosynthesis that occurs after formation of inflammatory tissue isoprenoid intermediates. The second biomarker, described earlier in this presentation, characterizes an individual's average daily dietary intake of EFA, predicts the likely intensity of n-6 eicosanoid actions during tissue responses and, most importantly, estimates the likely risk of death from coronary heart disease (*http://efaeducation.nih.gov/sig/personal.html*).

Primary prevention of these two dietary imbalances should begin in childhood *(10)* to prevent the two dietary imbalances and avoid much morbidity and mortality. To

design successful future dietary interventions that control EFA intakes, a simple interactive calculator (*http://efaeducation.nih.gov/sig/dietbalance.html*) can be used to predict probable proportions of n-6 in tissue HUFA by using the daily dietary intake of four types of EFA. Eating foods with less ω-6 and more ω-3 fats is likely to improve the general health of people in Western countries. Current epidemiological associations *(9)* (*see http://efaeducation.nih.gov/sig/personal.html*) combine with the empirical equation *(3)* (*see http://efaeducation.nih.gov/sig/hufacalc.html*) to predict that lowering intake of linoleate (18:2n-6) to 1 en% and raising n-3 HUFA intake to 0.5 en% might over time prevent most CHD mortality.

To help individuals identify and select specific food choices that can maintain their tissue proportions of n-3 and n-6 HUFA at a personally desired level, the calculator was combined with an interactive United States Department of Agriculture nutrient database of nearly 12,000 food servings. The interactive menu planning program is downloadable free from *http://efaeducation.nih.gov/sig/kim.html*, allowing individuals to make personalized menu plans that meet each person's preferences in taste and level of risk aversion. We have sufficient knowledge to plan effective primary prevention of CHD (and other inflammatory diseases), but we do not know the agencies willing and able to develop and implement effective prevention interventions rather than promoting pharmacological treatments only after disease is evident.

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6 ω-3 Fatty Acids and the Risk of Coronary Heart Disease

A European Union Clinical and Financial Impact Evaluation of the Columbus Concept

Jonathan D. Belsey

Abstract

Populations whose diet is low in ω-6 polyunsaturated fatty acids (PUFA) and relatively high in ω-3 PUFA are known to have a reduced risk of cardiovascular disease. Several clinical trials have examined dietary change as a therapeutic intervention and confirmed the benefit of this approach. This chapter presents the results of a meta-analysis that demonstrates that, in patients with established coronary heart disease, the risk of a recurrent event is reduced by 29% (95% confidence interval 6–47%) by the introduction of a diet with a low ω–6:ω–3 ratio. By appropriate crop and animal feed selection, it is possible to yield both plant and animal-derived fats that fulfil these dietary requirements, and which can be applied simply, and cheaply on a population-wide basis: the Columbus Concept. Given current approx annual management costs for myocardial infarction of ~€2.5 billion across the European Union, we estimate potential savings of around €570 million if such dietary change was universally instituted in high risk cardiovascular populations.

Key Words: Polyunsaturated fatty acids; ω-3; ω-6; diet; Columbus concept; coronary heart disease; meta-analysis.

1. INTRODUCTION

Coronary heart disease (CHD) is the commonest single cause of death in the European Union (EU), accounting for approx 800,000 deaths/yr. Although equal numbers of men and women are affected overall, amongst men more than 50% of these deaths occur in those aged under 75, compared with just 22% of female deaths occurring in this age group *(1)*. Indeed, it is this disparity in age of onset of CHD that is one of the major determinants of the differences in mean life expectancy observed between the sexes in all European populations. The recent extension of the EU to include many new Eastern European countries has served to highlight the issue further, as many of these populations face a rising, rather than falling CHD incidence, unlike most Western European nations.

Whereas a substantial volume of research has focused on potential pharmacological means of modifying this risk, there remains significant potential to achieve the same objective by means of lifestyle and particularly dietary modification. The association between dietary fat intake and the risk of CHD has been well established since the

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1950s *(2,3)*, and over the last 25 yr, this has been refined into an appreciation of the critical importance of the relative proportions of the constituent fatty acid subtypes in the diet *(4–6)*.

A number of epidemiological studies, carried out in different populations, have observed that societies that traditionally consume diets rich in oily fish and/or fruit and vegetables tend to have a low risk of CHD *(7–12)*. The most likely explanation for this association is the high level of ω-3 polyunsaturated fatty acids (PUFA) found in these dietary sources, in contrast with the patterns found in societies with higher levels of CHD, where saturated fatty acids (SFA) and ω-6 PUFA tend to predominate in the diet.

PUFA fall into one of two families: ω-6 or ω-3-named according to the position of the first double bond in the carbon backbone (*see* Fig. 1). Both ω-6 and ω-3 PUFA are essential for normal cellular function but, as no conversion between the two families is possible in humans, both types must be derived from dietary sources. In the case of ω-6, the principal source is the high linoleic acid content of corn oil, sunflower oil, and safflower oil, all of which are found in abundance in a typical Western diet *(13)*. Sources of ω-3 PUFA are less widespread, depending as they do on oily fish-derived eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) or vegetable derived α-linolenic acid (ALA) – in this case oils from flaxseed, soybean, rapeseed or walnuts—ingredients much less commonly found in a modern Western diet.

The consequence of this differing dietary availability is that approx 8 to 16 times as much ω-6 PUFA as ω-3 PUFA is consumed in a typical Northern European diet *(15–17)*. There is good evidence to suggest that, in primitive and preindustrial societies, where diets tended to be rich in gathered seeds and fish, this ratio was unlikely to be greater than 1–2:1 *(16)*. Given the observed association between high ω-3 intake and low-CHD risk, the potential for reducing coronary risk by returning to a less refined diet is clear cut. A working party of the National Institutes of Heath (NIH), operating under the auspices of The International Society for the Study of Fatty Acids and Lipids (ISSFAL) has therefore recommended that dietary modifications be made, such that the total dietary intake of ω-6 PUFA (linoleic acid) should be between 4.44 and 6.67 g/d, whereas ω-3 PUFA intake (ALA + EPA + DHA) should be at least 2.87 g/d, yielding a dietary ratio of 1.5–2.3:1 *(18)*.

1.1. The Columbus Concept

Achievement of this dietary goal within the context of a modern mixed diet is not straightforward at a population level. Given that a widespread shift to broadly wild food intake is unlikely to be achieved, we must re-examine ways of using modern plant and animal-derived fats. Based on the observation that wild game animals tend to have more favourable fatty acid ratios than related farmed animals, the Columbus Concept has demonstrated that the same effect can be created deliberately. By feeding animals and fowl with rations based on wild-type seed sources, meat and eggs with naturally low ω-3:ω-6 ratios can be derived.

Eggs thus derived have been commercially available for several years, with each containing around 600 mg of both ω-6 and ω-3 PUFA (ratio ~1). Conventional eggs, by comparison, contain around 600 mg ω-6 and only 60 mg ω-3 PUFA in each egg (ratio ~10). Recently, plant table oil and meat from monogastric animals (e.g., chicken and pork) with a similar ω-6:ω-3 ratio of ~1 have been released in Belgium for pilot market

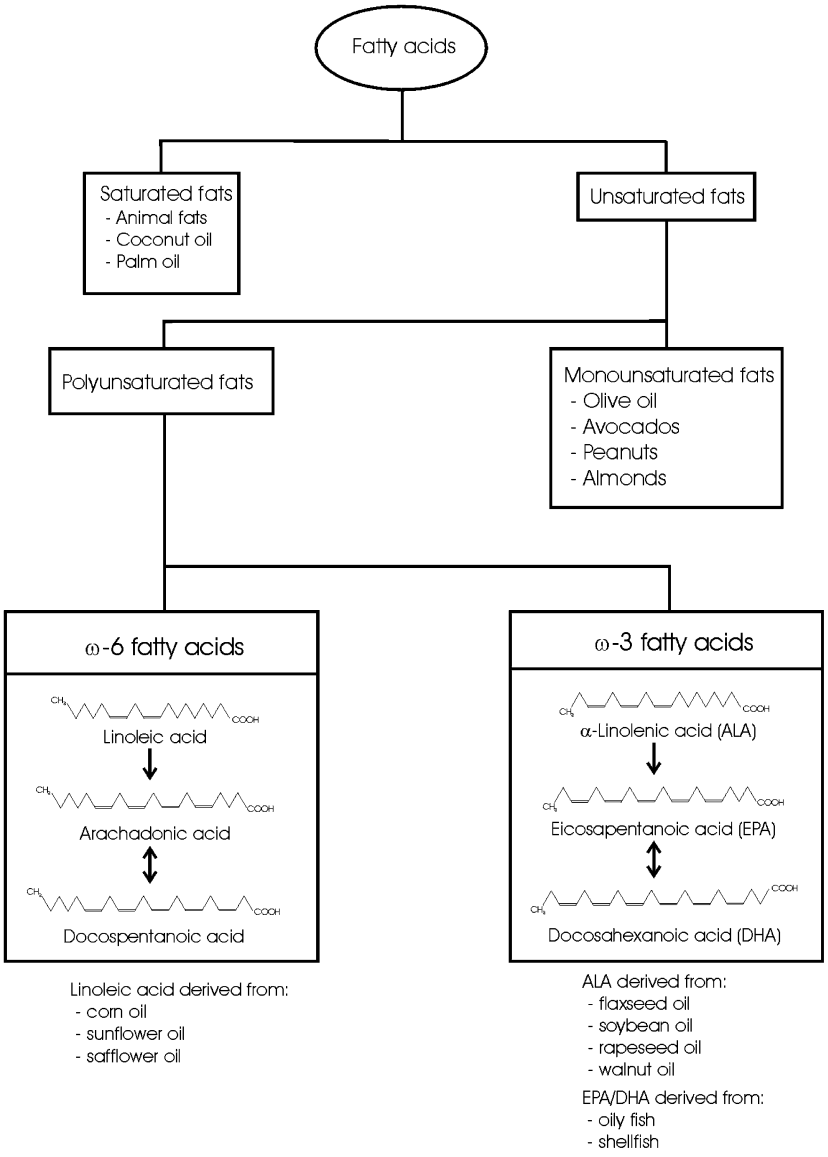

Fig. 1. Fatty acid subtypes. Adapted with permission from ref. *14*.

studies. Clearly, a tool like the Columbus Concept makes the ISSFAL PUFA targets considerably simpler to achieve, without requiring radical changes in population dietary habits or overall food expenditure.

However, we also need confidence that this approach will deliver a clinical benefit. For this an examination of the evidence base is required.

1.2. Evidence Base

Data from the Nurses' Health Study, based on 84,688 women, aged 34 to 59 yr, followed up for 16 yr, suggest that there is a quantitative and statistically significant relationship between the mean consumption of ω-3 fatty acids and the risk of

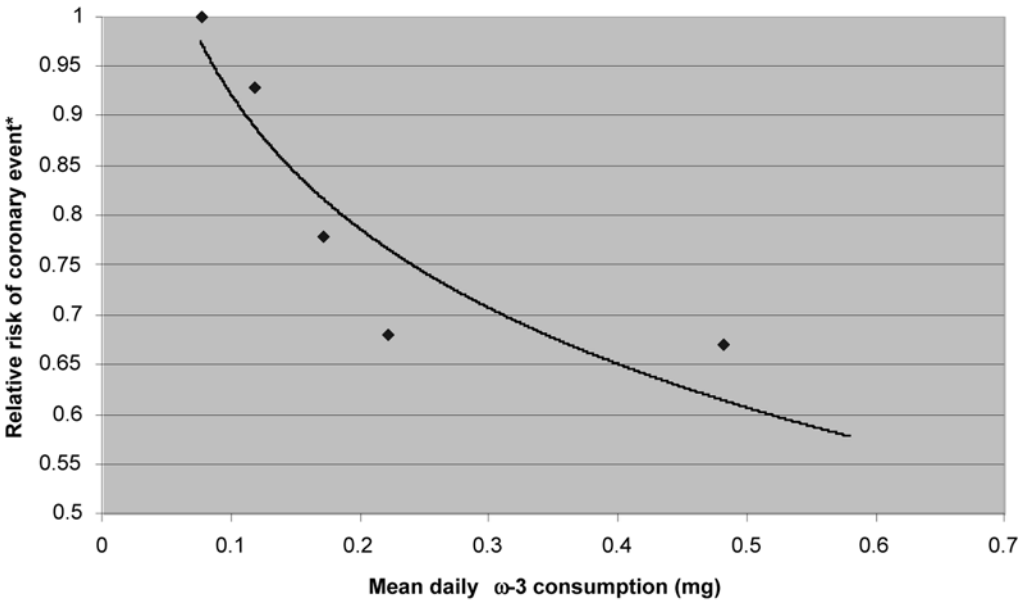

Fig. 2. Relative risk of coronary event in the Nurses' Health Study for each quintile of mean daily ω-3 consumption, compared to the baseline incidence in the lowest consumption quintile. Risks are adjusted for age, time periods, smoking status, exercise, hypertension, diabetes, hypercholesteremic, use of aspirin, oestrogens, multivitamins and vitamin E. Trend line is a logarithmic function R^2 = 0.837. *coronary event = first occurrence of non-fatal myocardial infarction (n = 1029) or death from coronary heart disease (n = 484). Adapted with permission from refs. *19* and *20*.

subsequent coronary events, especially amongst those individuals with a low baseline ω-3 consumption *(19,20)* (*see* Fig. 2). A smaller observational study, however, carried out amongst 44,895 men, aged 40 to 75 yr, followed up for just 6 yr, failed to demonstrate a similar dose-response relationship *(21)*.

There are, however, several problems inherent in using cohort studies to assess benefit. First, they may be very time consuming—because they tend to deal in relatively low-risk populations, many operate over time-scales measured in decades. Indeed, the lack of benefit observed in the Health Professionals' Follow-up Study *(21)* may simply reflect its short time scale. Second, regardless of the findings it is very difficult to presume causality, because of the risk of confounding factors. Although we know a great deal about the participants in the Nurses' Health Study and can control for these factors in our analyses, there may be other factors in the past medical or social history that are influencing the measured outcome and which are beyond the scope of the dataset. Third, and perhaps most importantly, dietary habits change. Although dietary patterns were assessed on 5 occasions over the 16 yr for the participants in the Nurses' Health Study *(19)*, the conclusions of the Health Professsionals' Follow-up Study appear to be based on a single baseline dietary assessment. Given that the participants were all doctors, it is entirely plausible that, between 1986 and 1992, dietary intake had changed *(21)*.

Thus, although cohort studies are useful tools for generating therapeutic hypotheses, it is only by looking at randomised controlled trials that we can quantify the magnitude of clinical benefit associated with ω-3 supplementation.

The objective of this chapter is therefore two-fold: (i) to carry out a systematic review and meta-analysis of randomized controlled trials relating to the clinical benefits of ω-3 supplementation and (ii) to evaluate the potential clinical and economic impact of this benefit, if it were to be implemented across the EU.

2. METHODS

A systematic review was carried out to identify relevant studies. Randomized controlled trials of ω-3 rich dietary interventions were identified using electronic searches of MEDLINE and EMBASE databases up to February 2004. The following text words were used in the search strategy:

omega-3		coronary		
OR		OR		
n-3		myocardial		randomized
OR	AND	OR	AND	OR
fish		infarction		randomized
OR		OR		
linolenic		reinfarction		
OR				
eicosapentanoic				
OR				
docosahexanoic				

The Cochrane Central Register of Controlled Trials was also searched using a similar approach. This was then combined with a manual follow-up of reference lists from primary papers and relevant review articles in order to pick up any studies not otherwise identified.

Studies were then included in the analysis if they fulfilled the following criteria:

- Randomized, single or double blind study control arm involving either dietary intervention or use of an ω-3 supplement.
- Primary outcome measure related to mortality and/or morbidity associated with coronary heart disease. Sufficient data presented to enable the assessment of the number of patients experiencing a fatal or nonfatal myocardial infarction.
- Data presented to enable an assessment of the difference in mean intake of ω-3 fatty acids between treatment groups.

A potential source of error in meta-analysis is the inclusion of data from studies which are, themselves, biased. In order to minimize this risk it is therefore important that some form of quality appraisal is carried out. For the purposes of this review, we chose to use a simple scoring system which has, itself, been rigorously appraised and validated to maximize inter- and intra-observer consistency *(22)*. Each included study was quality-appraised according to a five-point scale, with one point being awarded for each of the following criteria:

- Was the study randomized?
- Were details of randomization method given?
- Was the study blinded?
- Were details of blinding method given?
- Was information on study withdrawals given?

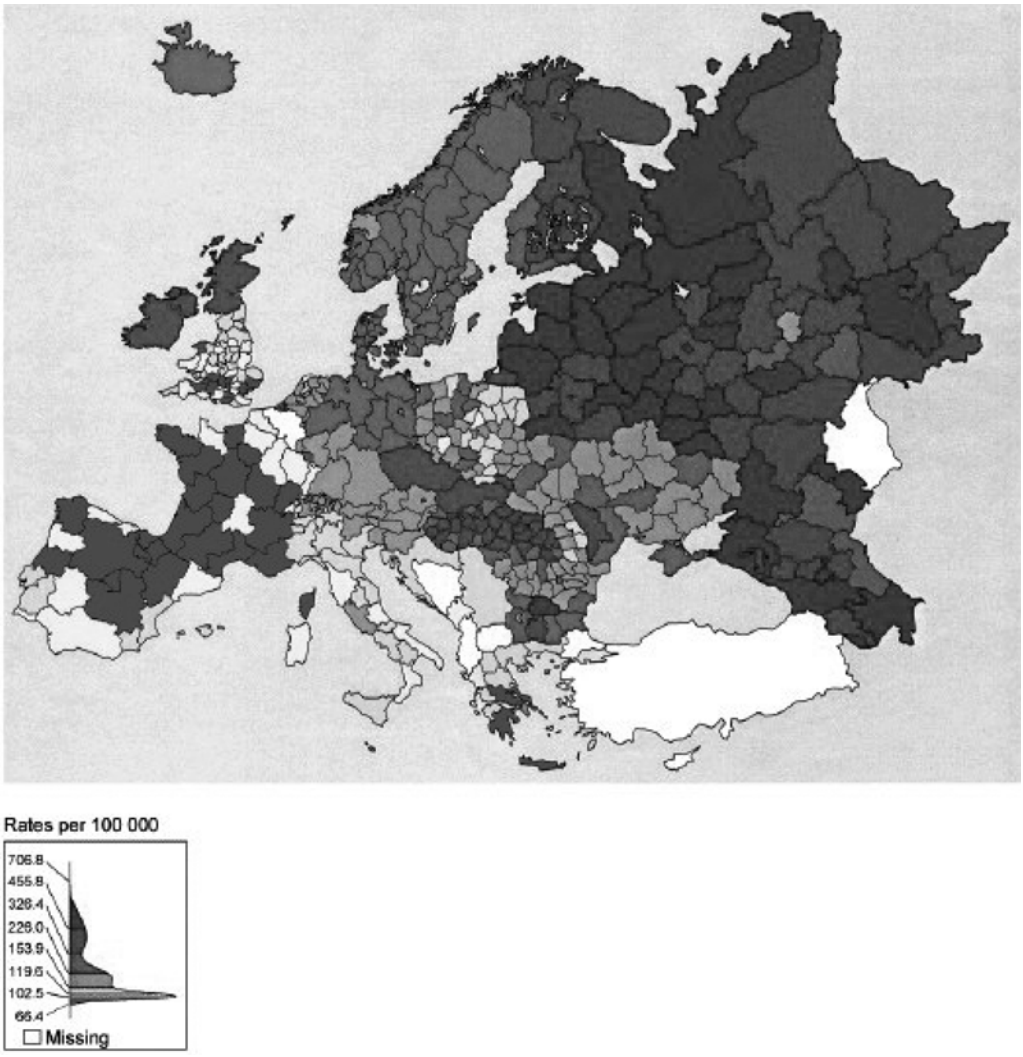

Fig. 3. Age-standardized mortality from ischemic heart diseases (ICD410-414) in European regions in 1990–1991 from men aged 0–64 yr. Reprinted with permission from ref. *23*.

Data relating to each triptan dose was pooled using a DerSimonian-Laird random effects model, in order to compensate for between-trials differences. Although results for this type of analysis are normally presented as odds ratios, in this case relative risks were pooled using the modified method defined in the Cochrane Collaboration Review Manager guide, in order to preserve the multiple risk factor adjustments presented in the original papers. Given that the event rates in these studies are generally low, it is unlikely that this procedure will have introduced significant error into the pooled estimate. Statistical significance was tested conventionally using the *t*-distribution for the logarithms of the pooled weighted estimates of the adjusted relative risks.

In order to test the potential treatment benefit of a change to dietary habits, a clinical impact model was then constructed, based on the following EU population groups, defined according to their geographical and epidemiological characteristics (*see* Fig. 3):

- **Group A**—*Northern and Western EU*: Austria, Belgium, Denmark, Finland, Germany, Ireland, Luxembourg, Netherlands, Sweden, UK. Total population: 198 million. This group are characterized by a high but declining incidence of coronary heart disease.
- **Group B**—*Southern and Western EU*: Cyprus, France, Greece, Italy, Malta, Portugal, Slovenia, Spain. Total population: 180 million, This group are characterized by a low and static incidence of coronary heart disease.
- **Group C**—*Eastern EU*: Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Slovakia. Total population: 72 million. This group are characterized by a high and rising incidence of coronary heart disease.

In each group, total coronary heart disease prevalence has been estimated by using mortality data. No systematic international method exists to record morbidity information in relation to nonfatal events and, although cause-of-death data are subject to a degree of subjectivity, fact-of-death data are consistently reliable. We know from a number of European sources that the case fatality rate for acute myocardial infarction is around 50%, including sudden deaths prior to hospital admission, *(24,25)* so it is a reasonable assumption that the prevalence of acute myocardial infarction will be approx double the number of deaths attributed to this cause. For the purposes of this model we have used the most recent mortality figures currently available, although many of these are now several years out of date *(26)*. Throughout this chapter, we have restricted our analysis to individuals between the ages of 45 to 85, in order to avoid both those patients with rare hereditary hyperlipidaemias and those cases where myocardial infarction is an inevitable terminal event in the very elderly.

2.1. Impact on Life Expectancy

Typically, following myocardial infarction, 25% of patients will die before reaching hospital, while total 30-d mortality will approach 50% *(24,25)*. This clearly has a major impact on the overall life expectancy of patients with CHD. Data from the Framingham Heart Study have been analyzed to identify the mean reduction in residual life expectancy for patients of different ages (Table 1) *(27)*.

These data were applied to each population under study, using age-sex specific myocardial infarction data from the UK *(28)* to estimate distribution of events overall, in order to arrive at an aggregate number of life years lost for each group.

2.2. Treatment Benefit

The magnitude of potential treatment benefit will depend on the baseline dietary patterns of the population group under study. Evidence from a number of sources suggests that current mean ω-3 consumption in typical Western diets is sufficiently low that it can be considered to be at "control" levels for our purposes *(13–17)*. Clearly, however, not every individual within a population has an inadequate diet. We have therefore assumed for the purposes of the model that 80% of the at-risk population in Groups A and C stand to gain from dietary modification.

It is more difficult to estimate, however, typical dietary intake in the Southern European group. Clearly a proportion of the population will be consuming a classical "Mediterranean" diet, already rich in ω-3 (and ω-9) fatty acids. In reality, however, many inhabitants of these countries will be consuming more conventional Northern European

Table 1
State-Specific Life Expectancy in Years by Age and Sex

	Health state		
Age/Sex	*Healthy*	*Post MI*	*Difference*
Males			
50	26.7	13.9	12.8
60	20.0	10.8	9.2
70	13.5	7.5	6.0
80	8.3	4.3	4.0
Females			
50	32.3	14.9	17.4
60	24.5	11.6	12.9
70	17.2	7.2	10.0
80	10.8	5.3	5.5

Adapted from ref. *27*.

diets. For the purposes of this analysis, we have assumed that 25% of the population in Group B potentially stand to gain from dietary modification.

Based on these assumptions, clinical benefit was estimated by applying the pooled estimate of relative risk reduction from the meta-analysis to the overall event rates of the three population groups, suitably scaled down according to the assumptions above.

2.3. Financial Impact

The heterogeneity of the data available from the published clinical trials makes a formal health economic assessment of population-wide ω-3 enhancement difficult to carry out and of doubtful validity. We have therefore presented an analysis of a defined sub-group—those patients with established cardiovascular disease—using a simple decision-tree approach. This secondary prevention model is based on the following clinical and cost assumptions which consider purely the major direct health costs over the course of 4 yr following an acute myocardial infarction (MI). This approach has been updated from a previously published financial impact analysis of a dietary intervention *(29)* and is based on UK 2002 treatment costs *(30)*, converted to Euros at a rate of €1.45 to £1.00.

1. Data relating to the acute management of myocardial infarction in Europe and reasonably up to date and enable us to model the first 30 d of treatment with a degree of confidence.
   a. 25% of patients experiencing an MI will die prior to medical help arriving *(25)*.
   b. A further 25% will die in the subsequent 30 d, yielding an overall case fatality rate of around 50% *(24,25)*.
   c. Between 20 and 25% of patients will have a revascularization procedure in the course of their admission *(32)*.
2. Data relating to long term service utilization, however is scanty. One publication draws on UK data from 1992 *(33)*. In this survey, of those surviving the first 30 d, over the subsequent 4 yr:
   a. 67% were readmitted to hospital for a cardiovascular reason.

b. 15% underwent coronary angiography.
c. 11% underwent revascularization procedures.

Given that use of both angiography and revascularization throughout Europe has increased 2- to 3-fold since 1992 *(34)* and that the UK has always been a relatively low user of both, it is likely that these are significant underestimates. We have therefore made an arbitrary assumption that the figures for angiography and revascularization should be increased to 40 and 30% respectively. This will remain an underestimate for high-using countries such as France and Germany but is likely to be a realistic mean figure for the extended EU as a whole.

The following cost assumptions were made *(30)*:

- A basic admission for a myocardial infarction costs €1595.
- An angiography costs a further €1595.
- A PTCA costs €3560.
- A coronary artery bypass graft costs €9100.
- The mean European ratio of PTCA:CABG is 1.9:1 *(34)*, therefore the mean cost of revascularization overall will be €4370.

2.4. Cost of Treatment

Given that this analysis concerns a governmental perspective on dietary modification, we have not assigned any treatment costs to this intervention. Whereas individuals may elect to use an ω-3 supplement to boost intake, the benefits being examined are entirely achievable by means of cost-neutral dietary change.

3. RESULTS

3.1. Meta-Analysis

The literature search identified six randomized controlled trials involving 7776 patients in 7 active treatment arms and 7674 patients in 6 control treatment arms *(35–40)* (Table 2). All patients in these studies were at high risk of having a myocardial infarction, either because of established ischemic heart disease or the presence of known coronary risk factors. Treatments included simple dietary modification *(35,36)*, augmented dietary modification *(37)*, or the use of low *(38,39)* or high dose *(40)* ω-3 supplements. Follow up ranged from 1 to 4 yr and in all cases data was available to assess the risk of fatal or nonfatal coronary events over the course of the study. Quality scores in all cases were 3/5 or greater, points generally being dropped for blinding issues. Data from all the studies were included in the final analysis. Where results had been corrected for potentially confounding variables by the original authors, these were preferentially included in the analysis, rather than recalculating unadjusted relative risks from raw data.

The results of the meta-analysis are summarized in Fig. 4. The pooled estimate of treatment effect was a relative risk of 0.71 (95% confidence interval [CI] 0.53–0.94, $p < 0.05$). The DerSimonian Laird estimate of pooled absolute risk reduction was 4.7% (95% CI:1.8–8.3%), yielding an estimated number needed to treat of 21 (95% CI:12–56). There is no suggestion of dose response apparent from these data. Indeed, the two studies that used the highest dose of ω-3 supplement (GISSI-Prevenzione *[39]* and Nilsen et al. *[40]*) appeared to yield the least treatment benefit.

Table 2
Summary of Included Studies

Study	*Patients*	*Intervention*	*duration*	*N (act)*	*N (con)*	*Quality*
DART *(35)*	♂ <70 Post MI	Dietary advice (high fish intake)	24 mo	1015	1018	3/5
Singh (Indo-Med) *(36)*	♂ ♀ (91%♂) All ages High CHD risk	Dietary advice (vegetarian ω-3)	24 mo	499	501	3/5
Lyon Heart Study *(37)*	♂ ♀ <70 Post MI	Dietary advice Special low fat spread supplied	46 mo	204	219	5/5
Singh (Acute MI) *(38)*	♂ ♀ All ages Acute MI	1. Mustard oil (2.9g ALA)	12 mo	120	118	5/5
		2. Fish oil (1.08 g EPA)	12 mo	122	118	
GISSI-Prevenzione *(39)*	♂ ♀ All ages Post MI	Fish oil ± vit E (0.85–0.88 g EPA)	42 mo	5666	5668	3/5
Nilsen *(40)*	♂ ♀ All ages Post MI	Fish oil (3.4–3.5 g EPA)	18 mo	150	150	3/5

3.2. Breakdown of Outcome

Although the primary outcome of the meta-analysis is a composite of endpoints, it is necessary to ensure the validity of the financial impact model to examine the relative impact of fatal and nonfatal events in the pooled outcome we have presented. Separation of the data and repooling reveals that, although clinical benefit is only independently statistically significant in respect of fatal coronary events (pooled RR = 0.66, 95% CI: 0.56–0.78, $p = 0.002$) with no independent significant benefit being seen for nonfatal myocardial infarction (pooled RR = 0.71, 95% CI: 0.46–1.06, $p = 0.08$), the two outcomes are associated with a similar magnitude of relative risk reduction, suggesting that it is valid to assume overall event rate reduction.

3.3. Clinical Impact

3.3.1. Group A—Northern and Western EU: Austria, Belgium, Denmark, Finland, Germany, Ireland, Luxembourg, Netherlands, Sweden, and UK

1. Total population: 198 million
2. Population ages 45–85 77 million (35.6 m male, 40.1 m female)
3. Annual deaths from CHD: 423,000 (218,000 male, 205,000 female)
4. Deaths from CHD (ages 45–85) 294,000 (174,000 male, 120,000 female)

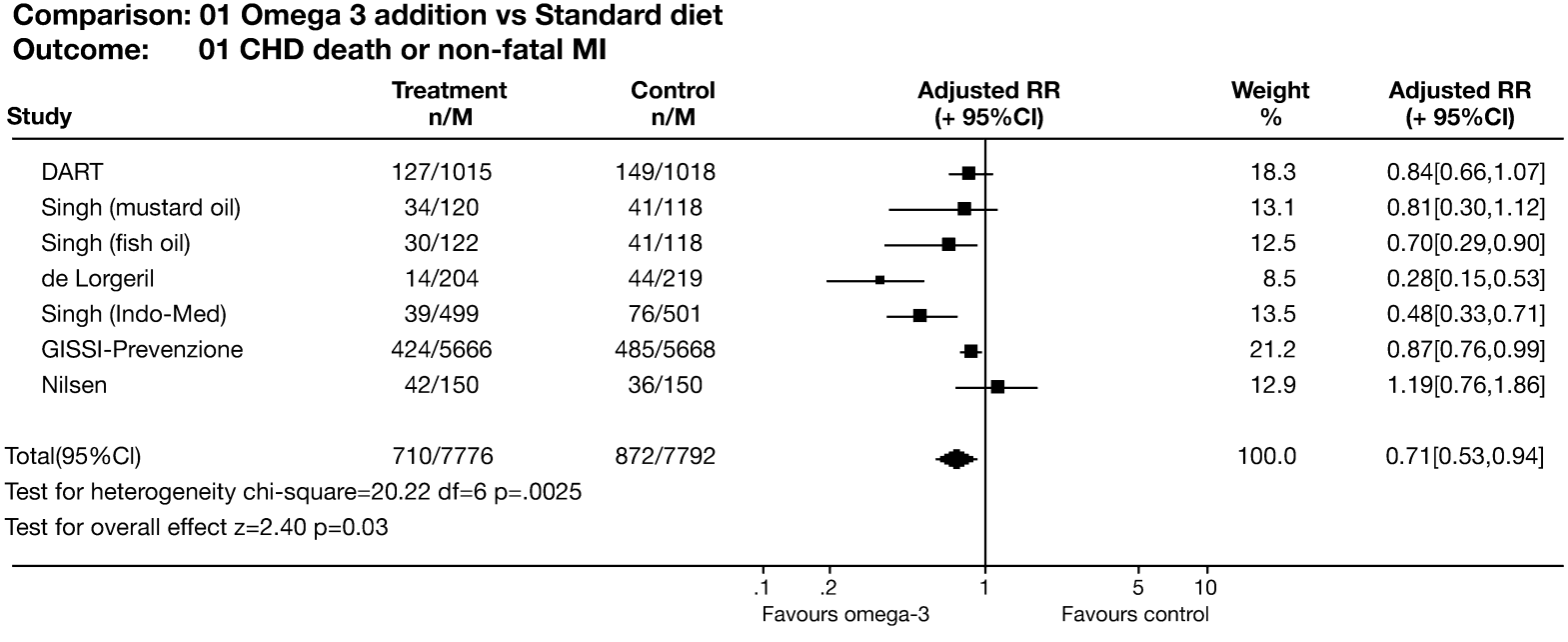

Fig. 4. Summary of results of meta-analysis of studies comparing ω-3 supplementation with standard diet in patients with established ischemic heart disease.

5. Estimated MIs (ages 45–85):	588,000 (348,000 male, 240,000 female)		
6. Event numbers by age/sex:		Male	Female
	45–54	23,600	5800
	55–64	55,000	18,800
	65–74	115,600	65,200
	75–85	153,800	149,400
7. Total life years lost:		Male	Female
	45–54	302,100	100,900
	55–64	506,000	242,500
	65–74	693,600	652,000
	75+	615,200	821,700
	Total	**3,934,000 life yr**	

3.3.2. Treatment Impact

Maximum potential impact assuming:

- 85% of individuals over 45 have inadequate dietary ω-3 intake.
- All these individuals adopt revised dietary strategy/supplements.
- Treatment is associated with a 29% reduction in coronary event rates overall.
- Benefit is equal across all age groups.

Results indicated:

- Number of myocardial infarctions prevented (age 45-85): **145,000 events**
- Number of life years saved: **970,000 life yr**

3.3.3. Group B: Southern and Western EU. Cyprus, France, Greece, Italy, Malta, Portugal, Slovenia, and Spain

1. Total population:	180 million		
2. Population ages 45–85	68 million (32.2m male, 35.8m female)		
3. Annual deaths from CHD:	184,000 (103,000 male, 81,000 female)		
4. Deaths from CHD (ages 45–85)	128,000 (82,000 male, 46,000 female)		
5. Estimated MIs (ages 45–85):	256,000 (164,000 male, 92,000 female)		
6. Event numbers by age/sex:		Male	Female
	45–54	11,100	2200
	55–64	25,900	7200
	65–74	54,500	25,000
	75–85	72,500	57,300
7. Total life years lost:		Male	Female
	45–54	142,100	38,300
	55–64	238,300	92,880
	65–74	327,000	250,000
	75+	290,000	315,150
	Total	**1,694,000 life yr**	

3.3.4. Treatment Impact

Maximum potential impact assuming:

- 25% of individuals over 45 have inadequate dietary ω-3 intake.
- All these individuals adopt revised dietary strategy/supplements.

- Treatment is associated with a 29% reduction in coronary event rates overall.
- Benefit is equal across all age groups.

Results indicated that:

- Number of myocardial infarctions prevented (ages 45–85): **19,000 events**
- Number of life years saved: **127,000 life yr**

3.3.5. Group C: Eastern EU. Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, and Slovakia

1. Total population:	72 million		
2. Population ages 45–85	27 million (12.8m male, 14.2m female)		
3. Annual deaths from CHD:	142,000 (76,000 male, 66,000 female)		
4. Deaths from CHD (ages 45–85)	100,000 (61,000 male, 39,000 female)		
5. Estimated MIs (ages 45–85):	200,000 (122,000 male, 78,000 female)		
6. Event numbers by age/sex:		Male	Female
	45–54	8300	1900
	55–64	19,300	6100
	65–74	40,500	21,200
	75–85	53,900	48,600
7. Total life years lost:		Male	Female
	45–54	106,200	33,100
	55–64	177,600	78,700
	65–74	243,000	212,000
	75+	215,600	267,300
	Total	**1,334,000 life yr**	

3.3.6. Treatment Impact

Maximum potential impact assuming:

- 85% of individuals over 45 have inadequate dietary ω-3 intake.
- All these individuals adopt revised dietary strategy/supplements.
- Treatment is associated with a 29% reduction in coronary event rates overall.
- Benefit is equal across all age groups.

Results indicated that:

- Number of myocardial infarctions prevented (aged 45–85): **49,000 events**
- Number of life years saved: **330,000 life yr**

3.4. Financial Impact

The clinical impact analysis suggests that, in the EU as a whole, the potential exists to prevent 213,000 coronary events annually, with a resultant saving of 1.4 million life years, by means of a simple dietary modification. Consequent financial savings are made by the health care purchaser, chiefly in terms of reduced management costs in year 1 after infarct. Figure 5 shows an example of how these have been assessed for the first year, this process then being re-iterated for the subsequent three years of the model. The financial impact model was as follows:

Group A
- Current annual cost of management: €1,440 million
- Cost if 85% used modified diet: €1,050 million
- Potential annual savings: €360 million

Group B
- Current annual cost of management: €630 million
- Cost if 25% used modified diet: €580 million
- Potential annual savings: €50 million

Group C
- Current annual cost of management: €492 million
- Cost if 85% used modified diet: €361 million
- Potential annual savings: €131 million

EU TOTAL
- Current annual cost of management: €2,562 million
- Cost if modified diet used: €1,991 million
- Potential annual savings: €571 million

4. DISCUSSION

The magnitude of clinical benefit associated with a reversion to an ω-3 rich diet is substantial and comparable to that seen with other well established pharmacological interventions, such as lipid lowering with statins *(41)* or use of antiplatelet agents *(42)*. Like these interventions, however, in order for a strategy to be both clinically and cost effective, it must be addressed at a population at sufficiently high risk of the outcome in question. In the case of this analysis, amongst population groups with a high baseline rate of CHD and low intake of ω-3 fatty acids, the potential for gain is impressive: in the case of both Northern and Eastern Europe the potential exists to save around 1200 life yr/100,000 population in the at-risk age group. In Southern Europe, however, where dietary patterns are substantially different and standardized morbidity rates are half those seen in the North, the potential for gain is substantially less, with the result that the level of clinical benefit demonstrated—190 life yr saved/100,000 population—is significantly lower. The magnitude of financial savings are commensurate with these clinical benefits.

These conclusions, however, are based on an assumption that is largely speculative: the level of ω-3 intake in a typical Southern European population. Our estimate that 75% of the population is already taking an adequate diet is based on no published evidence. It is, of course, entirely possible that the low level of CHD observed in these countries reflects other lifestyle or genetic factors, and that dietary differences are not as great as we imagine. If this is the case, we will have underestimated both the clinical and financial benefit associated with dietary modification in population Group B.

An interesting aspect of this analysis is that this intervention does not involve expenditure on the part of either health care purchaser or, necessarily, the consumer. In this respect, there are substantial differences from other cardiovascular interventions, where purchasers must usually make significant investment decisions in order to yield clinical and/or financial returns. Given that no clinical disbenefits have been identified, this inevitably means that the intervention will be cost dominant, regardless of baseline CHD rates and would therefore be equally applicable across all population groups in the EU.

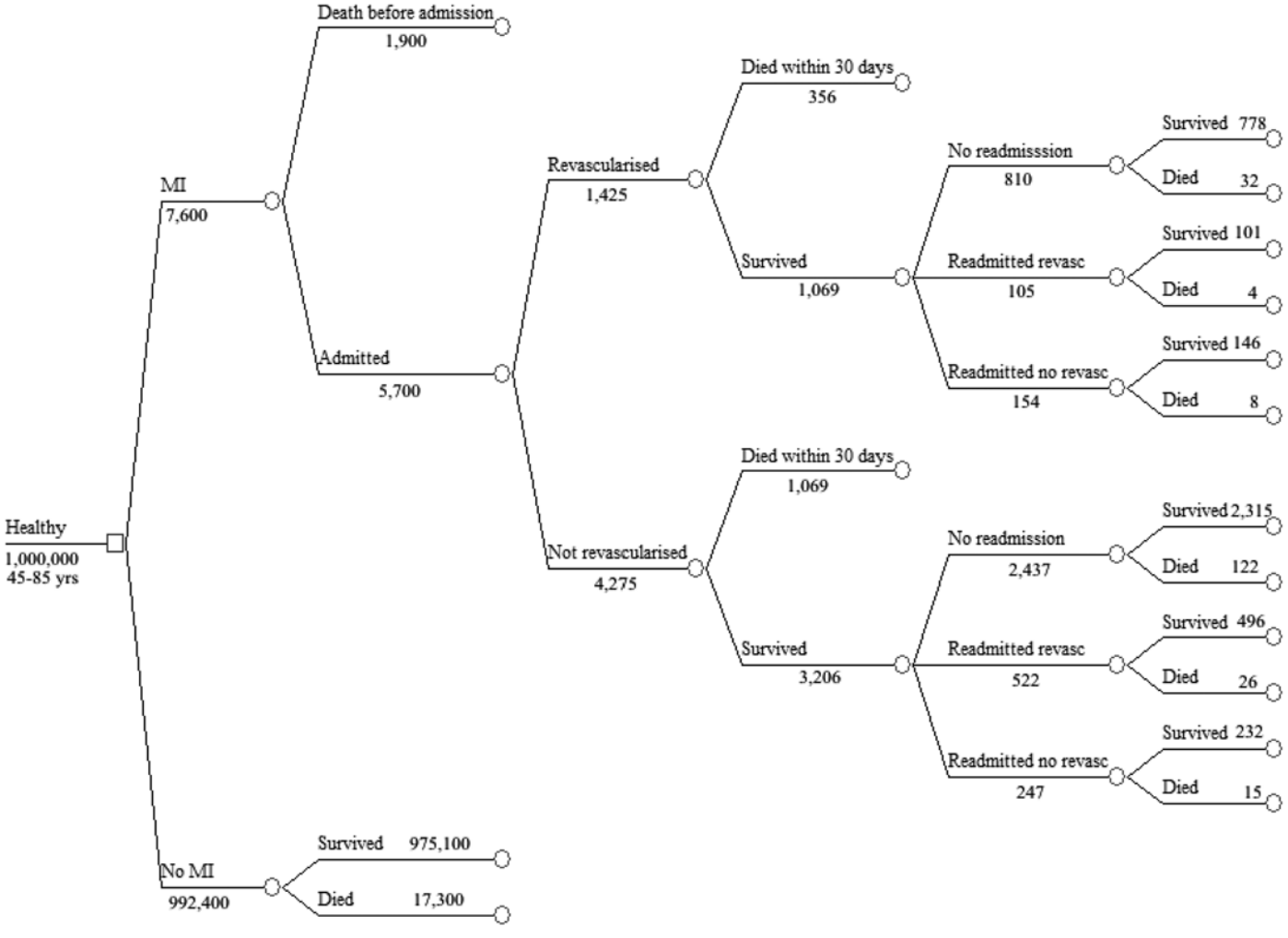

Fig. 5. Decision tree approach to estimating year one costs of myocardial infarction. Example from Group A prior to introduction of dietary change.

4.1. Fatty Acid Modification

Significant genetic change is unlikely to have occurred in metabolic capacity of humans over the course of the last few hundred generations. Over this period, however, our social structure has evolved from that of hunter-gatherer to a settled city-based society dependent on centralized food production. Ready access to farmed food sources has resulted in dramatic increases in overall fat intake with, in the past century, the familiar consequent effects on the incidence of atherosclerotic cardiovascular disease.

Whereas increasing awareness of this issue over the past 30 yr has not actually led to any significant decline in overall fat intake, there has been a significant shift from animal to plant sources, which now account for more than 50% of all fat in the Western diet *(44)*. This reflects a relatively unsophisticated health education message aimed at reducing population levels of total and LDL-cholesterol by shifting consumption from saturated to unsaturated sources. Unfortunately, because the majority of this fat is derived from cereals, spreads and fried foods, these are largely ω-6 sources, so the cardiovascular gain may not be as great as might have been anticipated.

By attempting to mimic the dietary sources of fats associated with a "wild-type" diet, it should prove possible to optimize the clinical gains that have been shown to be achievable in clinical trials *(36)*. It has been demonstrated, however, that our capacity to exert clinically significant change on the dietary behavior of populations is relatively limited *(45)* and it may therefore be more realistic to rely, instead, on modifying the fatty acid configuration of the foods already commonly encountered in a typical mixed diet—the Columbus® Concept. Although not a substitute for an otherwise healthy lifestyle, by ensuring that the plant and animal products consumed by the population have the optimum ω-6:ω-3 profile it becomes possible to maximize the cardiovascular gain in an essentially passive fashion, yielding both clinical and financial benefits, without detracting from any potential other gains that may be accrued from other personal or population interventions.

4.2. Study Limitations

Some aspects of the analysis may result in overestimates of benefit. We have made an assumption that dietary modification prevents both fatal and nonfatal events on the basis of comparable pooled relative risk reductions, despite the fact that independent statistical significance was only achieved for the fatal outcome. Among the component studies of the meta-analysis, two studies demonstrated a significant benefit for nonfatal myocardial infarction *(36,37)*, whereas the remainder did not *(35,38–40)*. This is a recurring problem in economic analysis, where composite and secondary outcomes must often be examined in a manner that was not envisaged when the primary studies were designed. The approach is widely used and probably acceptable, but we must nonetheless bear it in mind as a potential source of error.

The second aspect is that we have assumed a proportionate reduction in downstream interventions—PTCA, CABG—consequent on a reduction in the number of myocardial infarctions. Although this seems like a reasonable assumption, this aspect of CHD management has not been explicitly examined in any of the published randomized controlled trials.

Finally, we have excluded myocardial infarctions in anyone over the age of 85. This actually represents around 20% of infarcts in men and 40% in women. There is no *a priori*

reason to suppose that dietary modification would not help prevent many of these events. There were, however, very few people of this age group in any of the studies, so one cannot be certain as to the benefit. In addition, however, there is the question of diagnostic validity. Our morbidity estimates were based on mortality rates. Autopsy confirmed diagnoses, however, are rare in the elderly, and one must treat certified causes of death with a degree of caution, given their known limitations in the case of out of hospital cardiac deaths *(44)*.

5. CONCLUSION

There is a substantial, and generally convincing evidence base to support the cardiovascular benefits of a diet in which ω-6 and ω-3 PUFAs are equally well represented. Although this goal can be achieved by a switch to a wild diet that is rich in fats derived from ω-3 sources, it is perhaps unrealistic to anticipate such a population-wide change in diet. A viable and acceptable alternative involves the production of plant and animal products that possess a naturally favourable ω-6:ω-3 ratio, by virtue of their crop selection and feeding regime, respectively: the Columbus® Concept *(46)*. Such a strategy, if applied in populations with a high baseline level of coronary heart disease, offers the potential to achieve substantial clinical benefits in a highly cost effective fashion.

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7 The Nutritional Conditions of Human Evolution

Current Paleoanthropological Understanding

S. Boyd Eaton

Abstract

The human genome has changed only minimally since behaviorly modern humans appeared in East Africa between 100,000 and 50,000 years ago. Genetically, we contemporary humans remain adapted for the foods our ultra-great-grandparents were consuming then, and this insight should advance conventional nutrition science.

At that time and place the best current research indicates ancestral humans obtained about 30% of their food energy from protein, 35% from carbohydrate, and about 35% from fat. Cholesterol-raising saturated fats constituted perhaps 7.5% of total energy and harmful trans fats practically none. Polysaturated fatty acid intake was higher than at present and the ω6:ω3 ratio was closer to unity, say 2:1 -vs >10:1 currently. Cholesterol intake exceeded that at present. Carbohydrate came almost entirely from fruits and vegetables (honey provided about 2% of overall caloric intake vs the 15% added sugar consumed at present.) Fiber intake was high (~100 g/d) with a greater proportion of soluble fiber than is common currently. Ancestral vitamin and mineral intake (and probably phytochemical intake as well) generally ranged from two to five times current levels, the exception being sodium: Stone Agers consumed less than 1g each day, a third of current consumption.

Our ancestral dietary pattern deserves attentive consideration and investigation because of its theoretical logic and especially because established nutritional recommendations have failed to stem the rise of chronic degenerative diseases.

Key Words: Human evolution; evolution of human diet; dietary evolution; human nutrition.

1. INTRODUCTION

Humans living before the appearance of agriculture, in the Stone Age, were hunters and gatherers, for which another anthropological term is foragers. They are popularly referred to as "cavemen," even though they seldom lived in caves and certainly weren't all men. Whatever they are called, the people of that far-off time seem to evoke inherent interest among enthusiasts of all stripes, so it is understandable that a treatise on wild-type foods might contain a chapter describing what is currently known about the foods our remote Stone Age ancestors obtained during the many millennia before agriculture came into existence beginning about 10,000 years ago.

However, there is another reason for appreciating the nutrition that fueled nearly all human evolution: an increasing number of investigators believe the dietary patterns of

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our ancestors may constitute a guide to proper nutrition in the present. Contemporary dietary recommendations cover a wide range, running from the ultra-low-fat Pritikin and Ornish programs, through the more moderate Zone and South Beach plans, to the high-fat Atkins diet. And popular authors aren't the only ones whose recommendations vary all over the spectrum. Academic nutritionists writing in prestigious medical journals advocate a similarly wide range of nutritional regimens, from the low-fat East Asian pattern to the much more fat-liberal Mediterranean approach. These conflicting recommendations tend to confuse and even dismay health-conscious readers who frequently learn of research findings through simplistic and often sensationalized media accounts. Sometimes completely contradictory findings are headlined just a few years apart *(1)*. β-Carotene appeared to reduce cancer risk in initial studies, then seemed to increase risk in a later investigation *(2,3);* dietary fiber was first thought to reduce colon cancer susceptibility and then found to have no such effect *(4,5)*; sodium consumption has been linked to high blood pressure in many studies, but not in numerous others *(6)*. High fat diets are associated with coronary heart disease, but what about the "French paradox?" The French consume at least as much saturated fat as do Americans, but have considerably less coronary atherosclerosis.

In view of such inconsistencies, it is not surprising that advice varies. A logical, straightforward and understandable starting point from which to develop research protocols and upon which generally accepted recommendations may ultimately be based would be highly desirable. The ancestral human diet might provide such a foundation. Even though the Stone Age is far in the past, eminent paleoanthropologists, geneticists, biologists, and evolutionary theorists believe that human genes have changed hardly at all in the interim *(7)* . Although we live in the Space Age, genetically we are still Stone Agers. The genetic determinants of our current biology were selected not for contemporary circumstances, but for the conditions of life as experienced in the remote past. There are two potential corollaries *(8,9)*: first, that today's chronic degenerative diseases such as diabetes, many cancers, atherosclerosis (including coronary heart disease [CHD]), hypertension, osteoporosis, and obesity, are promoted by discord between our genes and our lives; and second, that the frequency of these illnesses might be greatly reduced by reinstating essential features of ancestral experience, including relevant nutritional practices, into our current lifestyle.

2. HUMAN EVOLUTION

Over the past half century, archeological finds in Africa and elsewhere have allowed paleoanthropologists to define the trajectory of human evolution with increasing confidence *(10)*. The common ancestor of both chimpanzees and humans is thought to have been a knuckle-walking African ape. Between 8 and 6 million years ago (mya), one of the first major developments in the lineage leading ultimately to humans was adoption of upright posture and bipedal locomotion. Australopithecines, the genus immediately ancestral to our own (Homo) appear in the archeological record something after 4.5 mya. They were better walkers than their predecessors although their arm structure suggests they were more agile tree climbers than we are now. Their brains remained small, much closer to those of chimpanzees than to ours and their overall body size remained similar to that of chimpanzees, substantially smaller than that of current humans.

A pivotal species, *Australopithecus habilis* (also called *Homo habilis*) appeared perhaps 2.5 mya. These human ancestors were the first to make stone tools and this technological advance gradually improved the efficiency of obtaining food. During the subsequent half million years, both body and brain size increased until by about 1.8 mya our ancestors, now called *Homo erectus*, had achieved the height of contemporary humans and their cranial capacity had enlarged from 400 cc, the figure for early australopithecines, to 900 cc. *H. erectus* originated in Africa, but subsequently migrated to other tropical and temperate parts of Eurasia, reaching Southeast Asia at least a million years ago and perhaps earlier.

Like their primate predecessors, the first Australopithecines were largely vegetarian and hunted small game (as chimpanzees do) when opportune, but obtained at least 95% of their caloric intake from vegetable (especially fruit) sources. However by the time Australopithecines attained the habilis stage, they appear to have scavenged. Perhaps the availability of stone tools helped them gain access to remains (brain, marrow) that were relatively inaccessible to carnivores. The gathering of aquatic foods (e.g., shellfish, turtles, frogs) from riverbanks and lake shores probably became routine *(11)* during this period. In addition to providing extra energy sources, the long chain polyunsaturated fatty acids available from scavenged brain tissues and aquatic foods may have been a contributing factor in subsequent human brain enlargement. Such fatty acids (HUFA) are structural components of gray matter hard for mammals to synthesize. Increased availability of these ready-made building blocks may have facilitated the brain expansion *(12)* that was an evolutionary selective advantage amid the ever-increasing complexity of ancestral social interactions.

As *Homo* species evolved they became increasingly capable hunters. Commingled spears and wild horse remains from 400,000 yr ago, found in what is now Germany, show that big game hunting was underway at that time; chemical analysis of Neanderthal skeletons indicates that these human cousins were prodigious carnivores. This is understandable in view of the subarctic environment that existed in Europe during the height of Neanderthal dominance there. As do Inuit (Eskimos), Neanderthals had minimal access to fruits and vegetables so that hunting was obligatory.

The earliest "anatomically modern" humans became recognizable in the anatomic record sometime after 200,000 yr ago. The skeletons of these early *Homo sapiens* are essentially identical to ours, especially those of us who are involved in habitual heavy physical exertion, like professional athletes. The lives of humans living before agriculture were intensely physical, hence their robusticity. However, despite their skeletal modernity, the lifeways of these near sapiens differed little, if at all, from those of earlier humans. Behaviorally modern humans appeared in Africa between 100,000 and 50,000 yr ago *(13)*. "Modern" behavior included relatively rapid and unprecedentedly innovative modifications of tools, weapons, and implements; expansion into areas previously uninhabited by humans (e.g., Australia ~50,000 yr ago; Siberia 30,000 yr ago; the Americas at least 12,500 yr ago); and replacement of earlier populations living in locations remote from Africa (e.g., European Neanderthals, residual *H. erectus* in southeast Asia) during this period. What led to behavioral modernity is debated, but the most widely accepted explanation at present is subtle brain reorganization allowing development of fully human speech with all the competitive advantage such improved communicative ability provided.

These early true humans were similar in most respects to hunter-gatherer groups studied during the past century. However, there were important differences. Recent hunter-gatherers have been increasingly restricted to infertile areas poorly suited to farming and where the biomass of wild animals, especially large game, had been much reduced. Also, recent foragers generally have some contact with nearby agriculturists which affects their culture to a greater or lesser extent. Recently studied hunter-gatherer groups have commonly been used as models for the preagricultural peoples of, say, 25,000 years ago and they *are* the best available surrogates, but the altered circumstances of otherwise similar people living many thousands of years apart need to be kept in mind.

3. ANCESTRAL FOODS

3.1. Plants

The vegetable foods available to foragers grew naturally, without cultivation, and included nuts, leafy vegetables, beans, fruits, flowers, gums, fungi, stems, and other similar items. These had been primate staples for tens of millions of years, but at some point along the hominid (human-like) evolutionary track, the digging stick came into use. This simple implement widened dietary breadth by providing access to roots, bulbs, and tubers—plentiful but previously inaccessible underground sources of food energy. The nutrient values of such foods naturally vary, but if several hundred representative vegetable foods utilized by hunter-gatherers studied during the past century are pooled and their averaged nutrient content then compared with the mean values for vegetable foods most commonly consumed in Western nations, several noteworthy differences emerge *(14,15)*. First, wild plant foods provide less energy per unit weight. A 100 g portion of the fruits and vegetables eaten by our ancestors would yield, on average, only about one third the calories that 100 g of contemporary vegetable food provide. This is primarily because so much of our current plant food intake is derived from high-energy cereal grains—rice, corn, wheat, and the like. Stone Age humans knew that grains were a potential food source, but given the technology available to them, the work required to process wild grains into digestible form was generally excessive compared with that needed to gather other types of wild plants for which processing was unnecessary or less arduous. Foragers generally viewed grains as emergency goods, to be used during times of shortage. It was only "late" in the human career, perhaps 30,000 yr ago in Australia but elsewhere (e.g., the Near East) between 15,000 and 10,000 yr ago, that evidence of routine cereal grain use became common.

Secondly, the nutrient content of wild plant foods is high, especially when the ratio of nutrients to calories is considered. While there is, of course, considerable individual variation, a mixed grocery bag of the fruits and vegetables available to ancestral humans would provide substantially more vitamins, minerals, and fiber than would a comparably representative collection of contemporary plant foods *(14,15)*. In many cases, vitamins and some minerals are artificially added to current foods, making them "enriched." This is less successful for fiber and not yet feasible for phytochemicals. The latter substances are plant constituents which influence bodily metabolic reactions. They can be considered semivitamins, but their total number is unknown and, for most, their mode of action is poorly understood. However, their importance for optimal health is becoming increasingly well established. Human ancestral biology became genetically adapted to the

phytochemicals provided by fruits and vegetables over hundreds to thousands of millennia. The phytochemicals of cereal grains, in contrast, are relative newcomers to human metabolism. Perhaps for this reason fruit and vegetable intake appears to reduce cancer susceptibility while consumption of cereal grain products has little or no such effect *(16)*.

Lastly, the plant foods available to ancestral humans afforded a more nearly balanced ratio of essential polyunsaturated fatty acids. Like essential amino acids, these cannot be synthesized by the body and must be obtained from the diet. They are necessary for cell membrane fabrication, especially in the brain, and they are also the basic molecules from which eicosanoids—a large class of important locally-acting hormones—are made. Essential fatty acids are divided into two families: omega-6 and omega-3. Both types are required for mammalian physiology, but they produce opposing biochemical effects, so roughly equal amounts in the diet are desirable. Unfortunately, in recent decades the use of safflower, corn, sunflower, and cottonseed for spreads and cooking oils has distorted the ratio. These materials contain 50 to 100 times more omega-6 than omega-3 so that, overall, Americans now consume 10 to 15 times more omega-6 than omega-3s *(17)*.

3.2. Animals

The wild game our ancestors ate differed in important ways from the commercial meat available at present. In the first place, today's meat is fatter. Whether the whole carcass or the most popular cuts (e.g., flank, loin, shank) are compared, commercial meat has up to four times more fat than does game. For example, 3.5 ounces (100 g) of regular hamburger provides 268 kcal, whereas the same amount of venison yields 126 kcal. Even when all visible fat is removed from a T-bone steak, the resulting separable lean portion contains 30% more energy than does game. These energy differences reflect the greater fat content of contemporary commercial meat *(15)*.

Not only is there more total fat, but the chemical composition of the fat in supermarket meat also varies from that in game animals. In general, fat from commercial meat has a higher proportion of saturated fatty acids (the kind which tend to raise serum cholesterol levels) than does the fat from game. Saturated fatty acids containing either 14 or 16 carbon atoms have been shown to have special propensity for raising serum cholesterol. Game fat typically has less of these substances when compared with an equal amount of fat from commercial meat. Another chemical difference involves the essential polyunsaturated fatty acids discussed earlier. The ratio of these in wild animal adipose tissue is much nearer equality than in most commercial meat. Grain feeding appears to be responsible: corn contains 50 times more omega-6 than omega-3 fat so the essential fatty acid composition of animals whose feed is based on corn becomes skewed, containing a far greater amount of omega-6 than omega-3 fatty acids *(17)*.

3.3. Other Considerations

3.3.1. Grains

Today cereal grains are "superfoods," not because of their nutrient properties, but rather because in many parts of the world members of the grain family provide from one-half to two-thirds or even more of the population's daily caloric intake. Rice in the Far East, corn in Mesoamerica, and sorghum in parts of central Africa are examples.

Such dependence on one or a few plant foods contrasts with the more broad-spectrum subsistence pattern of hunter-gatherers who commonly utilize one hundred or more types of food plants during the year. With limited exceptions, which probably didn't apply in the remote past, no one of these approaches the "superfood" status accorded cereals today.

Grains were infrequently used by ancestral humans because hand milling to render them digestible is such hard work that it wasn't energetically desirable to use grains unless other foods were in short supply. The situation changed when preagricultural population density reached the point that nomadism became impractical. When people were required to settle more or less permanently in a given area, grain consumption became a viable option because other types of plant and animal food became increasingly difficult to obtain. And it shortly became apparent that raising grains like wheat or barley could increase the total food energy available from a given geographical area. After farming got underway, population growth accelerated to rates greatly exceeding those typical before agriculture. On the other hand, individual health seems to have deteriorated. People became shorter while skeletal evidence of nutritional stress and infection became more frequent. Average life expectancy also appears to have declined, so the adoption of agriculture may not have been the societal boon it is often considered. In fact, Pulitzer Prize winner Jared Diamond has called it "the worst mistake in the history of mankind" *(18)*.

3.3.2. Dairy Foods

For most current humans, dairy foods are important constituents of each day's diet, but for free-living nonhuman mammals, mother's milk is the only "dairy product" ever consumed. After weaning, milk wasn't available for any primates, including humans, until the domestication of cows, goats, camels, and the like. Dairy foods have been an important component of official nutritional recommendations, at least in Western nations, since these first began to appear. Nevertheless, human ancestors, including behaviorally modern humans during four-fifths of their existence, thrived and evolved without any dairy foods whatsoever after they ceased breast feeding.

3.3.3. Alcohol

In the United States, alcohol provides about 1.3% of the average adult's daily caloric intake. It's not clear when the production of alcoholic beverages first developed, but most anthropologists doubt that wine, beer, mead, and especially distilled spirits were manufactured before agriculture. No hunting and gather groups studied in the 20th century were found to make such drinks.

3.3.4. Separated Fats

When ancestral humans consumed fats, they were generally obtained as integral components of whole foods; both animal and vegetable fats were coupled with the other nutrients intrinsic to the original source. In contrast, separated fats are staples for contemporary humans. Olive oil, butter, margarine, vegetable oils, lard, and the like are all vital ingredients for today's cooks. Such separated fats enhance our cuisine, but because fat provides about 9 calories/g (vs about 4 calories/g for protein and carbohydrate), the availability of fat in this form makes it possible to increase the energy density of our food in ways our ancestors couldn't.

3.3.5. Refined Flour and Sugar

Like separated fats, refined flours and sugars allow us to create foods with unnaturally high-energy density. In the worst case they are nearly pure energy—empty calories with few or no associated vitamins, minerals, or fiber. There are essential amino acids and essential fatty acids, required building blocks our bodies need to make necessary structural elements and required hormones. However, there are no essential simple carbohydrates like those provided by refined flour and sugar. Such carbohydrates are a convenient and efficient source of energy, but they offer little if any nutritional benefit over and above their caloric content. Fortified flours have additional nutrients food manufacturers consider desirable; our ancestors obtained their carbohydrate together with the nutrients Mother Nature selected.

3.3.6. Processed and Prepared Foods

Humans are the only free-living creatures to consume foods whose natural origins are obscure. Individuals unfamiliar with our culture would be unable to identify the ultimate sources of bread, pasta, sausage, cheese, and similar items which have been staples for millennia. Less traditional artificially fabricated foods have become immensely popular during the past century, to the point that for some of us such foods, often laced with gratuitous sodium, fat, and sugar, make up most of our daily intake. The list of ingredients found on the wrapper of almost any prepared food package affords one of the most telling commentaries on the differences between contemporary nutrition and that of preagricultural human ancestors.

3.3.7. Artificial Constituents

Organic food proponents would quickly point out that there are still other important differences between the naturally occurring plants and animals of 20,000 yr ago and most of those available to today's grocery shopper. Pesticides, hormones, fertilizers, antibiotics, dyes, and other additives are widely used in contemporary food production but weren't, of course, considerations in the remote past when humans ate exclusively "organic" food. The pros and cons of these modern innovations are debatable, but there is no question that they are "unnatural," and that humans evolved for millions of years before encountering foods like most of us eat at present.

4. OVERALL DIETARY PATTERNS

4.1. General Factors

There was no one universal Preagricultural diet. Our ancestors ate foods that were locally available and focused on those which returned the most food energy for the least expenditure of physical energy—a general rule for all biological organisms. Two important factors affecting diet choices were latitude and rainfall. In the savanna-like environment of northeast Africa, currently thought to have been the epicenter of human evolution, both game and vegetable foods were plentiful. Gathering plant foods in such circumstances was an integral aspect of the food quest for both males and females before our ancestors and those of chimpanzees diverged and for an uncertain length of time thereafter. At some point, most likely during the later stages of Australopithicine evolution, scavenging is thought to have become a significant component of hominid subsistence. It is not known whether this was an exclusively male function or whether

females participated as well. Because potential competitors for animal remains included hyenas and similarly dangerous beasts as well as the original predators, scavenging was little less hazardous than hunting, the main difference being the degree of technological expertise required. Later on, most likely for the past 500,000 yr and almost certainly since the appearance of behaviorally modern humans about 50,000 yr ago, obtaining food probably resembled the pattern observed among modern foragers, a division of labor according to gender with men hunting and women gathering.

Where they were relatively abundant, hunting large animals such as mammoths, red deer (similar to elk), horses, megamarsupials (some as large as rhinoceroses), and eland made energetic sense. More food energy can be obtained from one such carcass than from many smaller animals and the physical energy expended by the hunters is substantially less. Where large animals had become scarce, a variety of sophisticated techniques, including trapping and net hunting were used to increase the efficiency of obtaining small game. Along rivers where fish migrated seasonally (e.g., salmon runs) weirs and nets were used. In such locations Stone Agers sometimes lived year round, abandoning nomadism, establishing relatively large communities, and developing an early form of social stratification with elites—as opposed to nomadic hunter-gatherers who were almost always egalitarian.

Gathering was not confined to plant foods; women often brought home shellfish, eggs, small mammals, frogs, turtles, and the like. The process could be physically demanding. Women occasionally walked several miles, dug through hard ground (with a digging stick) to obtain roots or tubers, then walked back to camp carrying twenty to thirty pounds of foodstuff.

The relative contributions of hunting and gathering to forager economy are debated. Their respective importance almost certainly varied according to season and was surely affected by latitude. In the mammoth steppe of central Siberia, which was surprisingly well populated during the late Paleolithic of 30,000 to 10,000 yr ago, abundant wild grasses supported great herds of large game, especially mammoths, so that hunting flourished. However, edible plant food for humans was scarce. In this region hunting must have greatly exceeded gathering as a means of acquiring subsistence.

On the other hand, in East Africa, both game animals and wild plant foods were plentiful and in such areas hunting and gathering were more nearly equal in importance. Early studies of foraging groups inhabiting regions of this sort suggested that about two thirds of the food was obtained by gathering. However, more recent analyses now suggest that hunting and gathering made roughly equal contributions *(19)*. The new interpretation fits well with "optimal foraging theory," an anthropological law which merely formalizes the common sense observation that humans, like all other biological organisms, arrange their subsistence activities to maximize return relative to effort expended. When animals are plentiful and hunting techniques are well-developed, as seems to have been the case for the past hundred thousand years (and probably longer), the average returns from hunting exceed those from gathering. Nevertheless, gathering remained very important because even skillful hunters can experience unsuccessful periods, sometimes of uncomfortable duration. The practical botanical knowledge of foragers is so great that the women's success rate in finding plant food in fruitful regions approached 100% and many times tsi-tsi beans, baobab fruit, water lily roots and the like would have been our ancestors' only menu choices for dinner.

4.2. Macronutrient Ratios

Overall subsistence patterns in East Africa are of particular interest. If the "out of Africa" theory is correct, as seems increasingly likely *(13)*, what was routinely eaten in this region affected genetic adaptation in the direct ancestors of all living humans whereas foods encountered subsequently, as our ancestors spread throughout the world, had much less bearing on the contemporary human gene pool. The reconstructed nutritional patterns in this area around 50,000 yr ago are hence uniquely germane for those interested in the original "natural" human diet. With behavioral modernity came increasingly rapid cultural change, which has, to an ever-greater extent, substituted for genetic evolutionary adaptation. Dietary innovations such as the Mediterranean, East Asian, and vegetarian approaches to healthy eating have emerged too recently to have much effect on our genetic makeup. If there is a basic nutritional pattern to which humans are genetically adapted, the constituents provided by foods consumed in East Africa between 100,000 and 50,000 yr ago arguably define its nature *(20)*.

At this seminal time-place nexus, energy intake would have been higher than at present—probably about 3000 kcal/d for males and perhaps 2750 kcal/d for females. Because they lacked motorized equipment, draft animals, and most simple machines, caloric expenditure at this level was obligatory. In fact, it is likely that up until the early 20th Century energy expenditure and intake requirements remained substantially above those typical at present.

About 50% of the nutrients would have come from animal and fish sources whereas about 50%, on average, would have been of vegetable origin. Total caloric intake was likely partitioned about 30% from protein, 35% from carbohydrate, and 35% from fat. These estimates differ from the contemporary American pattern and also from current orthodox recommendations:

	E. African 50,000 BP	*Contemporary United States*	*Current Recommendations*
Protein	30%	15%	15%
Carbohydrate	35%	49%	55%
Fat	35%	35%	30%
Alcohol	–	1%	–

The differences are substantial and, at first glance, suggest that the Paleolithic diet was unhealthy. A little further analysis, however, is comforting for health-conscious paleoenthusiasts.

4.3. Fats

Our ancestors ate as much fat as we do. Muscle meat from game animals is very lean, but Stone Agers ate everything edible: marrow, brain, organ meat, and body fat from the thoracic and abdominal cavities, not just muscle as we tend to consume today. Optimal foraging means using the whole carcass. However, in contrast to fat from today's cattle, sheep, and pigs, the carcass fat of wild animals has substantially less serum cholesterol-raising effect. Most game fat is of the cholesterol-neutral monounsaturated variety, a substantial proportion is polyunsaturated, and much less is the saturated, cholesterol-raising type. Also, ancestral foods contained little to none of the cholesterol-raising *trans* fatty acids that

commercial hydrogenation adds to current diets. That hunter-gatherer diets are heart healthy is corroborated by the finding that the serum cholesterol levels of such people from around the world average below 130 mg/dL as opposed to about 200 mg/dL for Americans. And, although the available evidence isn't ideal (no coronary angiograms, few autopsies), as far as can be learned from clinical data, CHD is virtually unknown among them *(21)*. An additional factor that enhanced the heart-healthy nature of Paleolithic diets was their greater total intake (as much as ten times ours) and more nearly equal proportions of ω-6 and ω-3 essential polyunsaturated fatty acids. The great preponderance of ω-6 in contemporary Western diets is believed to be a factor contributing to our current cardiovascular disease epidemic.

4.4. Carbohydrates

Ancestral humans ate less carbohydrate than is typical for contemporary humans, the major difference being the near total absence of cereal grains and added sugar in their diets. However, the amount of fruits and vegetables consumed in areas resembling East Africa substantially exceeded that currently obtained in any part of the world and was more than double that typical in Western and Northern Europe. Much contemporary carbohydrate consists of refined flours and simple sugars—quickly absorbed and capable of inducing rapid rises in pancreatic insulin secretion. Stone Agers loved honey, but its availability was usually limited and seasonal (as indicated by their relatively caries-free dental remains). A far higher proportion of ancestral carbohydrate was in the complex form that had less adverse effect on insulin secretion *(20)*.

4.5. Protein

The levels of protein our ancestors consumed might once have been considered problematic, healthwise. However, early studies which attributed negative health effects to excessive dietary proteins now seem suspect. High-protein diets do aggravate kidney failure once it is established, but they don't appear to initiate the process. Autopsy studies of near traditional Inuit (Eskimos), whose protein intake was extremely high, did not reveal any extra kidney disease. It was once thought that high-protein diets were associated with colon cancer. In fact there is an association, but primarily because, in Western cultures, diets rich in meat provide excess saturated fat along with the protein and it is the former which now seems to foster development of colonic neoplasms, not the protein *per se*. High-meat diets were once thought to raise serum cholesterol levels, but again, associated saturated fat is the culprit. High-protein diets that contain little saturated fat actually lower serum cholesterol levels, an investigative result that might have been predicted based on findings among hunter-gatherers studied during the last century *(22)*.

4.6. Micronutrients

Americans and many others in affluent nations spend enormous amounts of hard-earned cash on vitamins (and, to a lesser extent, minerals) presumably in the hope that consuming such micronutrients may minimize the adverse effects of an otherwise unhealthy diet and lifestyle. Nutritionists usually decry this practice, arguing that micronutrient intake above and beyond RDA levels is unnecessary and that a balanced diet provides all the vitamins and minerals needed.

From a Paleolithic perspective, there is some virtue to both these views. Nutritionists follow Stone Age practice when they argue that it's better to obtain micronutrients from real foods rather than from capsules. However, ancestral micronutrient intake exceeded RDA levels in nearly every case (sodium was the principal exception). The greater total caloric intake necessitated by a physically vigorous lifestyle together with a micronutrient:energy ratio much higher for ancestral foods than for those commonly consumed at present means Stone Agers typically obtained from 1.5 to 5 times RDA levels of vitamins and minerals each day *(20)*. They didn't obtain anything like the recommendations of megavitamin enthusiasts, which can be up to 100 times the RDA in some instances.

Words like lycopene, anthocyanin, lutein, sulforaphane, isothiocyanate, and indole are beginning to appear regularly in popular articles on nutrition. These substances, and many others with equally unfamiliar names, are phytochemicals—vitamin-like molecules that affect our metabolism and biochemistry. The prefix "phyto-" indicates that they are constituents of plant foods. Those found in fruits and vegetables seem much more vital to human health than those from cereal grains *(16)*, presumably because our metabolism became adapted to the former over many millions of years as opposed to the few thousand years during which human biochemistry has routinely interacted with phytochemicals from cereals. The phytochemical content of uncultivated fruits and vegetables has been little studied, but it's likely that their phytochemical load would have paralleled their high content of known vitamins and minerals. Based on this supposition, plus the fact that Stone Agers in most areas consumed abundant quantities of fresh fruits and vegetables, it is probable that ancestral phytochemical intake exceeded that in the present.

Only 10% of the sodium consumed in Western nations is intrinsic to the basic foods themselves. The remainder is added during processing, preparation, and at the table. For our ancestors, as for all other free-living terrestrial mammals, potassium intake exceeded sodium intake, a circumstance almost certainly relevant to blood pressure regulation and to maintenance of cell membrane electrical potential. After salt became commercially available and especially as it became inexpensive, our diets have inverted the potassium:sodium relationship which characterized human and prehuman evolution, perhaps from the appearance of multicellular organisms over 500 million yr ago *(20)*.

4.7. Fiber

Since Denis Burkitt's research first drew public attention to the value of fiber in human diets, official recommendations for fiber intake have centered on about 20 to 30 g/d. However, our nearest nonhuman primate relatives, chimpanzees, consume about 200 g fiber/d. The fiber intake of ancestral humans would have been strongly influenced by the proportion of fruits and vegetables in their subsistence base because dietary fiber comes exclusively from plant foods. Stone Agers living at high latitudes, where edible vegetation is scarce, would have consumed even less fiber than we do at present. However, in East Africa, where modern human metabolism evolved, Paleolithic fiber intake is estimated to have been about 100 g/d *(20)*.

There are two main fiber types, both necessary for optimal human physiological function. Most plant foods provide some of each, but the proportions vary. Whole wheat and brown (unpolished) rice contain predominantly *insoluble* fiber, good for intestinal

tract function. Oats, corn, and most fruits and vegetables provide a high proportion of *soluble* fiber, valuable for regulating cholesterol absorption after meals. Modern, refined grain-centered diets have too little fiber generally, but, in addition, have a disproportionate amount of insoluble fiber. Preagricultural diets featuring more fruits and vegetables than at present provided a better balanced insoluble:soluble fiber ratio.

4.8. Acid Base Considerations

Ancestral diets tended to drive bodily pH toward alkalinity whereas modern diets have an opposite, acid-producing effect. Meat, cereal grain products, and dairy foods are the acidic elements, lowering pH, whereas fruits and vegetables are pH raising, alkalinizing agents. The body's homeostatic mechanisms ordinarily act to maintain pH at about 7.4, but over time (decades) the corrective metabolic measures necessary to offset persistent acid-yielding diets have pathophysiological consequences including calcium loss (which promotes osteoporosis) and kidney stone formation *(23)*.

5. CONCLUSIONS

The uncultivated plant foods and wild game that nourished ancestral humans and their prehuman predecessors were those to which our genetic makeup became adapted. Increasingly rapid cultural innovations during the past few thousand years have transformed our nutrition to the point that Cro-Magnons might not recognize many constituents of a typical meal: hot dogs (including the buns, mustard, and catsup), apple pie, and lemonade would all be unfamiliar. However, genetic evolution during the same period has been glacially slow, so that our genetically determined biology remains adapted for the literally natural and organic foods of the remote past. This dissonance between our genes and our lives has critical implications for our health, so an appreciation of ancestral nutrition should be of vital interest in the present.

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II Essential Nutrients in the Wild: Health Benefits

8 The "Wild-Type" Egg

An Empirical Approach to a Reference Pattern for Dietary Fatty Acids in Human Nutrition

Fabien De Meester

Abstract

Egg protein is recognized by the Food and Agriculture Organization and World Health Organization experts as the reference dietary source for essential amino acids in human nutrition. Their excellent digestibility and their high degree of similarity with the amino acid composition of body tissue protein make them outstanding among all other proteins from animal and plant origins.

Proteins and lipids contribute to most of the calories in egg and appear in a 1:2 ratio, similar to that recommended by modern dietary guidelines for human nutrition. Therefore, it is speculated that egg lipids can also serve as reference dietary source for essential fatty acids in human nutrition.

Taking 1999s National Institutes of Health Expert Committee's adequate intakes as a basis for the tentative establishment of a reference essential fatty acid pattern in human nutrition, it is shown that wild-type eggs outperform all other eggs in their ability to fulfill human (infants and adults) requirements for essential fatty acids. In addition, structural analyses predict that wild-type eggs are potentially antiatherogenic (e.g., normo-cholesterolemic and hypo-triglyceridemic) and are unique source of ω-3 fatty acids to body tissue. Preliminary clinical feeding trials confirm these theoretical expectations and the fact that the wild-type eggs represent an ideal vector of essential lipids to human and a unique platform for the establishment of a reference pattern for essential fatty acids in human nutrition.

Key Words: EEAs; EFAs; egg protein; egg lipids.

1. INTRODUCTION

The human organism finds its main source of energy in dietary carbohydrates, the real fuel of all biological life. In a balanced diet, carbohydrates account for 55% of the daily energy consumed by humans. These carbohydrates can themselves be turned to nonessential amino and fatty acids. However, a fraction of amino and fatty acids cannot be produced from carbohydrates and are therefore classified as essential amino acids (EAAs) and essential fatty acids (EFAs) in the sense they must be present in the diet in specific minimum amounts to fulfill tissues requirements.

A direct comparison between the energy contribution of proteins, lipids, and carbohydrates in table eggs and in a standard human diet provides evidence that egg is indeed an ideal balanced source of tissue-building macronutrients (proteins and lipids) and a very poor source of spare energy (carbohydrates) to human (Table 1). Proteins and lipids appear to contribute nearly a 1:2 ratio of the total energy of both table eggs and

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Table 1
Macronutrients in Table Egg and in Human Regimen

	Protein (amino acids)	*Lipid (fatty acids)*	*Carbohydrate*
% recommended	15	30	55
Cal/2000-Cal diet	300	600	1100
g/2000 cal diet	±75	±65	±275
g/100 g egg	±12	±10	±1

human diet whereas carbohydrates are almost absent in eggs. Basically, a 100 g ration of edible egg suffices to fulfill 15% of the human daily requirement for protein and lipid and does not contribute (<0.5%) to daily human energy expenditure.

Stated in a mathematical form, the tissue-building contribution of eggs to the human diet can be expressed as:

$$\frac{\text{Proteins}}{\text{Lipids}} = \frac{\text{Amino acids}}{\text{Fatty acids}} = \frac{1}{2}$$

This P:L ratio represents an optimal ratio, expressed in energetic terms (calories), between proteins and lipids for the build-up and maintenance of life in the animal kingdom (i.e., birds and men). It is interesting to note that all attempts to alter this ratio in table eggs have been deemed a failure. It is generally recognized that this ratio is genetically encoded (i.e., it is a result of Evolution in terrestrial animal life). From this perspective, it is sometimes hypothesized that table eggs represent an "ideal" or "optimum" in the sense that the ±50-cm^3 container basically contains, in perfectly balanced amounts—with no deficiencies and no surpluses—every single ingredient essential to the development of life in birds. As a corollary, table eggs stand as a nutritional standard for foods of animal origin. Does this mean that it is balanced for human nutrition purposes? Based on Darwin's theory and provided we can assume that man is an animal species evolved from an egg, one may indeed expect table egg composition to be in perfect balance with human needs in terms of nutrients from animal sources. Among macronutrients, these are proteins and lipids.

2. EGG PROTEIN

The 1990s Food and Agriculture Organization (FAO) and World Health Organization (WHO) has released a number of experimentally-accessible parameters that can be used to score the value of a dietary protein against human requirements (Table 2) *(1)*. Among them, the most useful parameters for comparison of dietary proteins in human nutrition are the "Net Protein Utilization" (NPU) and the "Protein Digestibility Corrected Amino Acid Score" (PDCAAS). The NPU is a measure of the degree of transfer of amino acids from the dietary protein to body tissue protein. It is thus dependent on the protein digestibility and the degree of similarity between the absorbed dietary amino acids and the body tissue composition. A NPU of 100% means that the dietary protein is perfectly fit for digestion and tissue incorporation, thus tissue-building and repair. The PDCAAS is a relative measure of the capacity of a dietary protein to support human requirements for EAAs compared with that of an ideal reference protein. A PDCAAS higher than

Table 2
Definition of Nutritional Quality Parameters for Dietary Proteins

Parameters	*Definition*	*Nutritional meaning*
TPD[a] True Protein Digestibility	Nitrogen absorbed from food × 100% / nitrogen ingested = (I – (F – F_k)) / I × 100%	TPD measures the fraction of the dietary protein that is absorbed from the diet, usually determined by measuring the amount of nitrogen in the food (I) and the amount excreted in the faeces (F), corrected for endogenous nitrogen losses in the faeces after a protein free diet (F_k).
TBV[b] True Biological Value	Nitrogen used for tissue formation × 100% / nitrogen absorbed from food	TBV measures the amount of protein that is retained from the absorbed protein. Essentially TBV measures the fraction of the nitrogen in the diet that is left in the body after nitrogen losses in the faeces and the urine have been substracted, correction made for endogenous nitrogen losses in the faeces and the urine after a protein free diet.
NPU Net Protein Utilization	TPD × TBV / 100	NPU expresses the ratio of the nitrogen used for tissue formation to the amount of nitrogen ingested and therefore can be calculated by multiplying TPD with TBV. NPU is also frequently quoted in the literature on biological assessment of protein quality.
PDCAAS[c] Protein Digestibility Corrected Amino Acid Score	(Ratio between content of most limiting amino acid in protein and reference protein) × TPD/100	PDCAAS gives the capacity of a protein to supply a person with an essential amino acid, taking into account its amino acid score against the reference protein and the true digestibility of the dietary protein.
PER Protein Efficiency Ratio	Weight gain (g)/g of protein intake in young rats	PER measures the ability of a protein to support growth in young rats. It represents the ratio of weight gain to the amount of protein consumed, under standardized conditions and diet containing 9.09% protein.

[a]The "apparent protein digestibility" or "PD" is the value obtained when correction for endogenous nitrogen losses is omitted.
[b]The "apparent biological value" or "BV" is the value obtained when correction for endogenous nitrogen losses is omitted.
[c]The "amino acid score" or "AAS" is the value obtained when correction for protein digestibility is omitted. Definitions are from ref. *1*.

Table 3
Nutritional Quality Parameters for Dietary Proteins

Parameters	*Beef*	*Cow milk*	*Hen egg*	*Soy*	*Wheat*
TPD[a]	98.0%	95.0%	98.0%	95.0%	91.0%
TBV[b]	80.0%	91.0%	100%	74.0%	54.0%
NPU	78.4%	86.5%	98.0%	70.3%	49.1%
PDCAAS (infants)[b]	0.70	0.75	0.90	0.54	0.37
PDCAAS (all ages)[b]	0.92	1.21	1.18	0.91	0.42

Infants: <1 yr.
[a]Data from ref. *35*.
[b]Data from ref. *36*.
Note: NPU = TPD × TBV.

1 characterizes a protein that outperforms in terms of its EAA composition for human nutrition; such a protein would have the capacity to rebalance a varied diet made of other underperforming proteins.

Table 3 shows the NPU and PDCAAS for a number of selected dietary proteins from animal and plant origins—including milk and egg—for infant and all other age feeding. One can see that egg protein surpasses all others in terms of its biological utilization in humans. With a NPU of 98%, egg stands as the FAO/WHO's reference in human nutrition. The next best choice for humans are other animal proteins followed by plant proteins. Both PDCAASs for infants and all other ages show that hen-egg protein is only slightly surpassed (PDCAAS: 0.90) by mother's milk for infant feeding and that it shares the pole position with cow milk for all other ages. The next best choices of protein for human feeding are those from animal sources: cow milk (NPU: 86.5%) and beef (NPU: 78.4%) followed by plant proteins, soy (NPU: 70.3%), and wheat (NPU: 49.1%). The fact that the NPU of hen-egg protein is higher than that of cow milk, although the two proteins have similar AASs, is another indication that egg protein not only outperforms in terms of its EAAs content, but that it also comes within a perfect environment for maximum biological utilization of its amino acid pattern.

The requirements of the human body for each individual essential amino acid have been determined *(2)*. The reference protein composition is taken from breast milk for infants (below 1-yr of age) and for all other-ages it is obtained through balance studies "which determine the minimum intake of each EAA (mg amino acid/kg body weight/d) at the point where total nitrogen intake equals total nitrogen excretion and the nitrogen excretion is minimal." At the present time, the pattern for 2- to 5-yr-old preschool children has been chosen as the reference pattern for all other ages because it is the most demanding in terms of protein needs of any age group other than infants. There currently is not sufficient data to differentiate between age groups. Based on these requirements the FAO and WHO adopted a theoretical reference protein pattern that optimally matches EEA requirements in humans older than 1 yr. In the reference pattern, the requirements are expressed in mg/g protein and are obtained by dividing the requirement (mg amino acid/kg body weight/d) by the safe level of protein intake (1.1 g protein/kg body weight/d) as established by the FAO and WHO (note that the minimum requirement is estimated at 0.75 g protein/kg body weight/d).

As seen in Tables 4 and 5, egg protein outperforms all other proteins from animal and plant sources with the former being much more appropriate for human nutrition than the latter. Table 4 shows that egg protein is very similar in quality to that of breast milk and would actually surpass it if not for a minor deficiency in leucine (Leu) which exhibits an AAS of 0.92 and explains the PDCAAS (TPD × AAS) of 0.90 (Table 2). Next to egg, we have cow-milk protein with an AAS of 0.79 for Met + Cys and 0.82 for Trp, beef protein with an AAS of 0.71 for Trp, 0.87 for Leu, 0.91 for Val and 0.95 for Met + Cys, and then the plant (i.e., soy and wheat) proteins with deficiencies in almost all EAAs.

Table 5 provides ample evidence that animal protein (i.e., egg, milk, and meat) are best suited for EAA requirements in humans above 1 yr of age. All three sources have an AAS above 1 (1.27 for Trp in cow milk, 1.21 for Lys in egg, and 1.09 for Trp in beef) whereas soy and wheat are suffering from a shortfall in Met + Cys (0.96) and Lys (0.46), respectively. Based on the recommended safe level of protein intake given above, one can calculate that a 70-kg adult can theoretically cover his needs for EAA through the intake of no more than 77 g/1.18 (PDCAAS) = 65 g egg protein or, based on 12.5 g protein/100 g edible egg, of 520 g edible egg (130 g whole egg powder).

It is interesting to note that more than 50% (ΣEAAs: 512 mg/g protein) of amino acids in egg protein are essential to humans and that 20% (ΣBCAAs: 206 mg/ g protein) of them are branched-chain amino acids (i.e., Ile, Leu, Val). Recent placebo controlled trials have shown that higher level of BCAAs in the diet results in decreased perceived exertion and mental fatigue during submaximal exercise *(3)*. Clinical observations have identified glutamine (Gln) as an efficient amino acid to reduce protein losses and infection during stress *(4,5)* and to speed up the glycogen recovery in the muscle *(6)*. As a tentative conclusion, one can say that egg protein stands as the FAO and WHO standard because it mirrors human body tissue protein composition. This conclusion is interesting from an evolutionary standpoint because the egg contains within itself all the essential ingredients for the development of life in birds. We can therefore turn to egg lipids and logically ask the same question: do egg lipids perfectly fit human needs?

3. EGG LIPIDS

The assessment of egg lipid nutritional quality is not straightforward because of a lack of standard in egg fatty acid composition, a lack of understanding in the relationship of the distribution between dietary and tissue lipids and, therefore, a lack of the FAO and WHO nutritional standards in human nutrition. This observation on the absence of standards in egg fatty acids holds true for all animal-derived lipids for which the fatty acid composition is to a large extent dependent on the type of fatty acids present in the feed of the breeding animal. In turn, the absence of fatty acid standards in animal nutrition has probably rendered difficult the interpretation of results of clinical and epidemiological studies carried out in the last quarter of the century and has no doubt contributed to the inception of the long-lasting biased diet-heart idea *(7)*.

In the absence of internationally recognized standards in human nutrition, 1999s NIH Expert Committee *(8)* has tentatively released adequate intakes (AIs) for infants and adults (Tables 8 and 9): for infants, these AIs are based on the averaged fatty acid pattern of breast milk from women in Europe and Africa (Table 6) *(9)* and are expressed in

Table 4
Amino Acid Score for Selected Dietary Proteins According to FAO/WHO Recommendation for Infant Feeding

Essential amino acid	*Breast milk WHO AA*[a]	*Beef*[a]		*Cow milk*[a]		*Hen egg*[a]		*Soy*[b]		*Wheat*[b]	
		AA	*AAS*	*AA*	*AAS*	*AA*	*AAS*	*AA*	*AAS*	*AA*	*AAS*
His	*26*	*34*	*1.31*	*27*	*1.04*	*22*	*0.85*	–	–	–	–
*Ile	46	48	1.04	47	1.02	54	1.17	47	1.02	33	(0.72)
*Leu	93	81	(0.87)	95	1.02	86	**(0.92)**	85	(0.91)	68	(0.73)
Lys	66	89	1.35	78	1.18	70	1.17	63	(0.95)	27	**(0.41)**
Met + Cys	42	40	(0.95)	33	**(0.79)**	57	1.36	24	**(0.57)**	39	(0.93)
Phe + Tyr	72	80	1.11	102	1.42	93	1.29	97	1.35	78	1.08
Thr	43	46	1.07	44	1.02	47	1.09	38	(0.88)	29	(0.67)
Trp	17	12	**(0.71)**	14	(0.82)	17	1.00	11	(0.65)	11	(0.65)
*Val	55	50	(0.91)	64	1.16	66	1.20	49	(0.89)	43	(0.78)
ΣEAAs	460	480	–	504	–	512	**–**	414	–	328	–
ΣBCAAs	194	179	–	206	–	206	**–**	181	–	144	–
AAS	1.00	–	0.71	–	0.79	–	**0.92**	–	0.57	–	0.41

Note: AAs (amino acids) are in mg/g protein; values within brackets are below infant requirements; bold values are for most limiting amino acid.
[a]Data from ref. *36*.
[b]Data from ref. *37*.

Table 5
Amino Acid Score for Selected Dietary Proteins According to FAO/WHO Recommendation for "Other Age" Feeding

Essential amino acid	*Balanced studies WHO AA*[a]	*Beef*[a]		*Cow milk*[a]		*Hen egg*[a]		*Soy*[b]		*Wheat*[b]	
		AA	*AAS*	*AA*	*AAS*	*AA*	*AAS*	*AA*	*AAS*	*AA*	*AAS*
His	*19*	*34*	*1.79*	*27*	*1.42*	*22*	*1.16*	–	–	–	–
*Ile	28	48	1.71	47	1.68	54	1.93	47	1.68	33	1.18
*Leu	66	81	1.23	95	1.44	86	1.30	85	1.29	68	1.03
Lys	58	89	1.53	78	1.34	70	1.21	63	1.09	27	**(0.46)**
Met + Cys	25	40	1.60	33	1.32	57	2.28	24	**(0.96)**	39	1.56
Phe + Tyr	63	80	1.27	102	1.62	93	1.48	97	1.54	78	1.24
Thr	34	46	1.35	44	1.29	47	1.38	38	1.12	29	(0.85)
Trp	11	12	1.09	14	1.27	17	1.55	11	1.00	11	1.00
*Val	35	50	1.43	64	1.83	66	1.89	49	1.40	43	1.23
ΣEAAs	339	480	–	504	–	512	–	414	–	328	–
ΣBCAAs	129	179	–	206	–	206	–	181	–	144	–
AAS	1.00	–	1.09	–	1.27	–	1.21	–	0.96	–	0.46

AAs (amino acids) are in mg/g protein; values within brackets are below "other ages" requirements; bold values are for most limiting amino acid.
[a]Data from ref. *36*. (The WHO AA reference pattern for all-ages is taken as the pattern for pre-school-children (2- to 5-yr-old.)
[b]Data from ref. *37*.

Table 6
Essential Lipid in Human Milk Compared With 1999 NIH's and FAO/WHO's Recommendations

mg/100 mL	*LA*	*γ-LA DHLA*	*AA*	*α-LnA*	*EPA*	*DPA DHA*	*CHN*	*CHL*
Human Milk[a]	350	13.65	11.70	26.32	1.30	12.65	14.5	18.0
NIH 1999	380	–	19	57	<3.8	13.3	–	–
FAO/WHO 1994	400	–	26.7	33.3	–	13.3	–	–

Note: NIH (1999) has released adequate intakes (AIs) for infant formula/diet, expressed in % of total fatty acids. The numbers in the table have been obtained based on averaged fat content of 3.8 g/100 mL in human milk. FAO/WHO *(36)* recommendations for essential fatty acids are given in mg/kg body weight or BW. The datain the table have been obtained based on averaged infant consumption of 150 mL milk/kg BW/d.

[a]Data from ref. *9*.

Table 7
Essential Fatty Acids in Modern, Non-Confined and Wild-Type Egg

Fatty acids (in 50-gm)	*Modern US-egg (grain-based)*	*Modern EU-egg (grain-based)*	*Ampelistra egg (olive-based)*	*Wild-type egg (grass-based)*	Δ
Ref.	a	b	a	b	
ω6	541	825	368.3	615	
ω3	27.7	91.7	282.6	598	
P	568.6	916.7	650.9	1213	+100%
S	1290.4	1245	1610.6	1178	–20%
P:S	0.44	0.74	0.40	1.03	
ω6:ω3	19.5	9.0	1.30	1.03	
M	50%	48%	50%	42.75%	–20%

Note: In mg per 50-gm edible egg. Δ is the difference between wild-type egg and Ampelistra egg. Ref. (a) from *12*, (b) from *13* and *14*.

percentage of total fatty acids; for adults, they are based on reviews of epidemiological and clinical studies and are expressed in mg/d for 70 kg sedentary adults on a 2000 cal diet.

Based on the total lipid content in breast milk (3.8 g/100 mL) and the FAO and WHO recommendation of 30% daily energy intake (65 g for 2000 cal) as fat for adults, we can calculate a theoretical reference EFAs pattern for infants and adults, expressed as mg EFAs/g dietary lipids. Tables 8 and 9 show the EFA requirements in humans approaches 120 mg/g dietary fatty acids and infants essentially differ from adults by their higher needs for ω-6 fatty acids (i.e., LA, AA) and apparent lower need for ω-3 fatty acids (i.e., ALA, EPA, DHA). This difference probably results from the fact that ω-6 and ω-3 fatty acids behave as natural growth promoters in infants whereas ω-3 fatty acids have a more regulatory role in adults and the elderly. A second observation is that 7.5 and 15% of all EFAs in infant and adult nutrition, respectively, are long-chain polyunsaturated fatty acids (LCPs) and the need for LCPs in humans seems therefore to be increasing with age.

The 1999 NIH Expert Committee's recommendations also tends to aggregate toward a "wild-type" standard where monounsaturated fatty acids would contribute to at least

Table 8
Fatty Acid Score for Egg Lipids According to 1999 NIH Recommendation for Infant Feeding

Essential Fatty acid	*1999 NIH FA*[a]	*1999 NIH FA*[b]	*M-US FA*[c]	*M-EU FA*[c]	*A FA*[d]	*W FA*[c]	*M-US FA*[e]	*M-EU FA*[e]	*A FA*[e]	*W FA*[e]	*M-US FAS*	*M-EU FAS*	*A FAS*	*W FAS*
LA	10	100	418	715	256	570	83.6	143	51.2	114	0.84	1.43	0.51	1.14
AA	0.50	5	80.3	85.2	86.4	34	16.1	17	17.3	6.8	3.2	3.4	3.46	1.36
Σω6-LCP	0.50	5	122.7	110	112.3	45	24.5	22.0	22.5	9.0	4.90	4.4	4.50	1.80
ALA	1.50	15	8.3	27.7	110.4	490	1.66	5.5	22.1	98	0.11	0.37	1.47	6.53
EPA	<0.1	<1.0	–	0.6	19.2	12	–	0.12	3.8	2.4	–	>0.12	>3.8	>2.4
DHA	0.35	3.5	17.4	43.3	105.6	78	3.48	8.7	21.1	15.6	1.0	2.49	6.02	4.45
Σω3-LCP	0.45	4.5	19.4	64.0	172.2	108	3.88	12.8	34.4	21.6	0.86	2.84	7.64	4.80
ΣEFA	12.35	123.5	568.6	916.7	650.9	1213	113.7	183.3	130.2	242.6	0.92	1.48	1.05	1.96

Notes: FAs (fatty acids) are in [a]percent of fatty acids, [b]mg/g dietary lipid (3.8 g lipid/100 mL milk), [c,d]mg/egg (50 g edible portion), [e]mg/g egg lipid (5 g lipid/egg). Numbers appearing in bold are for limiting fatty acids.

[a]adequate intakes (AIs) from 1999 NIH.

[c]data from ref. *14*.

[d]Data from ref. *12*.

Abbr: ΣEFA = LA + Σω6-LCP + ALA + Σω3-LCP; M-US: American modern egg; M-EU: European modern egg; A: Ampelistra egg; W: wild-type egg; FAS: fatty acid score;

two-thirds of the fat derived contribution to energy, with saturated and polyunsaturated fatty acids and, among the latter—ω-6 and ω-3—not substantially deviating from balance *(10,11)*. This recent hypothesis toward the most appropriate distribution of fats in the human diet (above 1 yr of age) is thus based on the scientifically irrefutable fact that modern man is genetically identical to his hunter-gatherer ancestors whose diet was characterized by a naturally balanced lipid ratio; an observation which in a mathematical form reduces to:

$$P{:}S = \omega 6{:}\omega 3 = 1{:}1$$

The fatty acid pattern of modern (M: grain-based), Ampelistra (A: olive-based), and wild-type (W: seed-based) eggs are given in Tables 7, 8, and 9. The modern egg is the standard, from hens fed a modern-type ω-6 rich grain based mash feed, available everywhere; the P:S ratio is relatively low (0.44 in the United States [US], 0.74 in the Eurpoean Union [EU]) and the ω6:ω3 ratio is sharply deviating from unity (19.5 in the US, 9.0 in the EU). The Ampelistra egg *(12)* is from the Southern Peloponese of Greece where hens roam within olive trees and eat spare fruits and leaves as well as grass, insects, and worms. Compared with their modern counterpart, these eggs have similar levels of saturated, monounsaturated, and polyunsatured fatty acids (P:S = 0.40; M: 50%), but present a close-to-balance ω6:ω3 ratio (1.30) similar to that of wild-type eggs (Columbus® egg, Belovo S.A.; *www.belovo.com*) from hens maintained in cages or in nonconstrained environments and fed an exclusive vegetarian diet whose lipid composition is similar to that of grass and greens and to that of a wild bird's diet *(13,14)*. Both P:S and ω6:ω3 ratio's in the wild-type egg approach unity and the egg contains twice the amount of polyunsaturated and 20% less saturated and monounsaturated fatty acids than the Ampelistra egg.

Based on the modern set of the 1999 NIH Expert Committee's recommendations and the "wild-type" hypothesis, one may expect that wild and wild-type eggs would perfectly fit the human need for EFAs. In an attempt to assess the nutritional quality of the lipid fraction of modern eggs and wild-type eggs against the 1999 NIH Expert Committee's adequate intakes for infants and adults, an assay was made to present the data in a similar format as that described by FAO and WHO for EAA requirements; therefore, EFA patterns of modern and wild-type eggs (mg fatty acids/g egg lipid) were computed and compared with the reference patterns for infants and adults described here above.

For infants (Table 8), it is seen that wild-type eggs (W) have no limiting fatty acid (fatty acid scores all above 1) and show up as a good source of ω-6 fatty acids (LA: 1.14; AA: 1.36; Σω6-LCP: 1.80) in combination with loads of ω-3 fatty acids (ALA: 6.53; EPA: >2.40; DHA: 4.45; Σω3-LCP: 4.80) which have been shown to be crucial to the development of brain and retina in babies and perhaps to avoid mental disorders (i.e., dyslexia, dyspraxia, ADHD, schizophrenia, autism) at a later stage *(15–17)*. Modern eggs (M) present a FAS of 0.11 (US) and 0.37 (EU) resulting from a deficiency in ALA compared with breast milk, and exhibits lower—although not limiting for the European standard—scores for ω-3 LCPs in general (EPA: >0.12; DHA: 2.49; Σω3-LCP: 2.84) compared with wild-type eggs. Ampelistra eggs (A) show a limiting FAS of 0.51 for LA, but otherwise exhibit excellent scores for LCPs of both families (Σω6-LCP: 4.50; Σω3-LCP: 7.64); in particular, Ampelistra eggs are the richest in EPA (FAS: >3.8).

Table 9
Fatty Acid Score for Egg Lipids According to 1999 NIH Recommendation for Adults

Essential fatty acid	*1999 NIH FA*[a]	*1999 NIH FA*[b]	*M-US FA*[d]	*M-EU FA*[c]	*A FA*[d]	*W FA*[c]	*M-US FA*[e]	*M-EU FA*[e]	*A FA*[e]	*W FA*[e]	*M-US FAS*	*M-EU FAS*	*A FAS*	*W FAS*
LA	4,400*	68	418.2	715	256	570	83.6	143	51.2	114	1.23	2.10	**0.75**	1.68
ALA	2,200	34	8.3	27.7	110.4	490	1.7	5.5	22.1	98	**0.05**	**0.16**	**0.65**	2.88
EPA	220	3.4	–	0.6	19.2	12	–	0.12	3.8	2.4	–	**0.04**	1.12	**0.71**
DHA	220	3.4	17.4	43.3	105.6	78	3.48	8.7	21.1	15.6	1.02	2.56	6.21	4.59
Σω3-LCP	650	10	19.4	64.0	172.2	108	3.88	12.8	34.4	21.6	**0.39**	1.28	3.44	2.16
ΣEFA	7,250	112	568.6	916.7	650.9	1213	113.7	183.3	130.2	242.6	1.01	1.64	1.16	2.17

Notes: FAs (fatty acids) are in [a]mg/d (2000 kcal diet); [b]mg/g dietary lipid (65 g lipid/d); [c]mg/wild-type egg (50 g edible portion); [e]mg/g egg lipid (5 g lipid/egg). Numbers appearing in bold are for limiting fatty acids.

[a]adequate intakes (AIs) from 1999 NIH.

[c]Data from ref. *14*.

[d]Data from ref. *12*.

Abbr: ΣEFA = LA + ALA + Σω3-LCP; M-US: American modern egg; M-EU: European modern egg; A: Ampelistra egg; W: wild-type egg; FAS: fatty acid score; *Upper limit in adults for LA: 6,600 mg; for AA: not specified.

In adults, egg lipid's FASs for wild-type eggs (Table 9; W) succeed with flying colors in all EFAs; only EPA is a bit low (FAS: 0.71) as compared with the 1999 NIH Expert Committee recommendation, but this apparent shortage disappears when all ω3-LCPs (EPA + DPA + DHA) found in wild-type eggs are taken into account (FAS: 2.16). Modern eggs (Table 9; M) show substantial deficiencies in ω-3 fatty acids of plant origin (ALA: 0.05 (US) and 0.16 (EU)) and of animal origin (EPA: 0.04) whereas Ampelistra eggs (Table 9; A) are relatively poor in the two EFAs of plant origin (LA: 0.75; ALA: 0.65), but are an excellent source of ω3-LCPs (EPA: 1.12; DHA: 6.21; Σω3-LCP: 3.44). All in all, the wild-type dietary lipid distribution seems to be in excellent agreement with modern recommendation for dietary intakes of EFAs in humans and in overall better agreement than their modern (M) and Ampelistra (A) counterparts.

In conclusion, and for as far as the basis of the fatty acid profile assessment developed in this paper is confirmed, one can predict that wild-type egg and wild-type animal-derived foods (i.e., egg, milk, meat) are characterized by more substantial lipid composition for human nutrition (i.e., their lipid make-up is in better compliance with human genetic heritage, provides sufficient ω-6 fatty acids, and supplies high amounts of beneficial ω-3 fatty acids). For infant feeding, it has been shown that EPA may compete with AA in the early stages of growth and development *(18–20)*. It would therefore be interesting to test the wild-type egg for compliance in infant feeding based on its characteristic fatty acid profile including both EPA and AA.

4. STRUCTURE-FUNCTION RELATIONSHIP IN EGG LIPIDS

4.1. General Considerations About Dietary Lipids

Dietary and tissue fatty acids appear to be linked to biological carriers such as cholesterol esters (CEs) and glycerol moieties (i.e., triglycerides [TGs] and phospholipids [PLs]). As these carriers are partially hydrolyzed during digestion and absorption, there is a relative degree of equivalence between the various carriers (i.e., CEs, PLs, TGs) and between the various positions (sn-1, 2, 3) on the carriers where a specific fatty acid can be found (*see* Fig. 1). During digestion, these natural carriers are hydrolysed by specific digestive enzymes (i.e., cholesterol esterase, sn-2 phospholipase, and sn-1/3 lipases) into free fatty acids (FFAs), free cholesterol (CHL), lysophospholipids (sn-1:LPLs), and monoglycerides (sn-2:MGs) (Table 10) (*see* Fig. 1). Except for short- and medium-chain fatty acids, which follow the portal vein gateway to the liver, the absorbed hydrolyzed lipids are then rebuilt within the enterocytes into CEs, PLs, and TGs, packed into chylomicrons (85% TG, 1% CHL, 3% CE, 9% PL), and released into the blood circulation through the lymphatic system. From this perspective, it is seen that dietary fatty acids located on CEs—at positions sn-1,3 of TGs and at position sn-2 of PLs—are nutritionally equivalent because they can be exchanged during digestion and resorbtion. Most of these fatty acids become available for tissue incorporation through their release by the sn-1/3 endothelial lipases lining the lymphatic vascular system and can thus be referred to as tissue-directed fatty acids (TFAs). Dietary fatty acids located at the sn-2 position of dietary TGs and at the sn-1 position of dietary PLs are not released during digestion nor are they incorporated into tissues during the chylomicrons' journey to the liver; rather, they are eventually reprocessed into postprandial lipoproteins in the liver. Therefore, dietary fatty acids located at these positions can be described as dietary messengers to

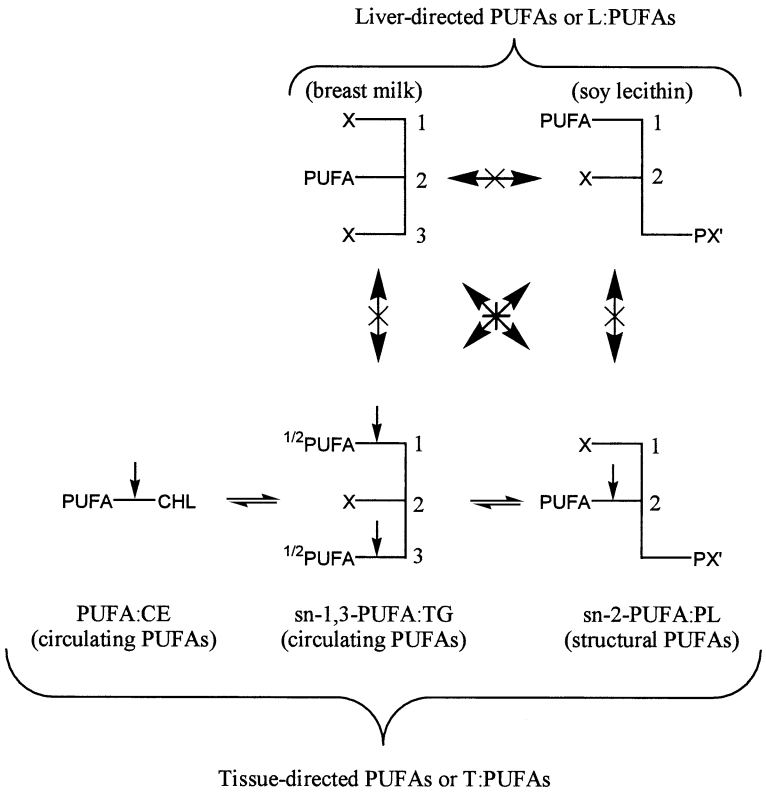

Fig. 1. Liver- and tissue-secreted dietary PUFAs. Most dietary fatty acids happen to be associated to lipophilic carriers such as cholesterol esters (CEs), triglycerides (TGs), and phospholipids (PLs). Structurally, there are three and two nonequivalent positions on the TG- and the PL-carriers, respectively. Dietary PUFAs available for direct tissue incorporation are those found as CEs and those located at sn-1,3 positions of TGs and at sn-2 positions of PLs. These positions are equivalent in terms of their nutritional value because they can and actually are exchanged during digestion and tissue/organ deposition and excretion. In this chapter, they are referred to as "tissue-directed PUFAs or TPUFAs." Dietary PUFAs found at other location (sn-2 position of TGs such as it happens in breast milk and at position sn-1 of PLs such as it occurs with soy lecithin) are deemed to find their way to the liver where they have some down-regulatory activities on lipogenesis at the genetic level. Dietary TGs with PUFAs at their sn-2 position and dietary PLs with PUFAs at their sn-1 position have been shown to exhibit hypolipidemic properties. In this chapter they are referred to as "liver-digested PUFAS or LPUFAs."

the liver and be referred to as liver-directed fatty acids (LFAs). The message brought to the peripheral tissues and liver by these dietary fatty acids have been studied and reviewed by Keys *(21)* and Hegsted *(22)* and depend on the nature of their carbon chain, with the general trends as follows: saturated fatty acids (SAFAs) are hypercholesterolemic, monounsaturated fatty acids (MUFAs) are rather normo-cholesterolemic, and ω6-polyunsaturated fatty acids (PUFAs) are hypocholesterolemic. A note of caution should be made here with regard to the hypocholesterolemic effect of dietary PUFAs, which seems to result solely from a transient or temporary accumulation of cholesterol in tissues and liver triggered by linoleic acid (LA)—but not by ω-3 fatty acids such as

Table 10
Dietary Fatty Acids Processing During Digestion

Dietary lipids	*Digested lipids*
CEs	CHL + FFAs
TGs	sn-2:MGs + sn-1/3:FFAs
PLs	sn-1:LPLs + sn-2:FFAs

Note: Upon digestion, dietary fatty acids in cholesterol esters (CEs), triglycerides (TGs) and phospholipids (PLs) give, respectively, free cholesterol (CHL) and free fatty acids (FFAs), sn-2 monoglycerides (sn-2:MGs) and sn-1 and sn-3 released free fatty acids (sn-1/3:FFAs), and sn-1 lysophospholipids (sn-1:LPLs) and sn-2 released free fatty acids (sn-2:FFAs).

ALA, EPA and DHA *(23)*—and which tend to cancel out in the long run *(24)*. Within the PUFAs, ω-3 exhibits strong hypotriglyceridemic properties. In fact, the only two requirements for a dietary fatty acid to be active as an hypolipidemic nutrient appear to be its location at position sn-2 of TGs or sn-1 position of PLs and a double bond at position n-6 on the carbon chain. Indeed, MUFAs are not hypotriglyceridemic, whereas ω6- and ω3-PUFAs as well as conjugated linoleic acids (CLAs) are *(25)*.

4.2. Consequences for Egg Lipids

Based on this structure-function relationship for dietary lipids, it is interesting to analyze the structure of egg lipids with the view of predicting their functions, assuming that egg fatty acids can be divided into two groups based on the fact that they are directed to peripheral tissues (TFAs) or to the liver (LFAs).

4.2.1. Cholesterolemic Effect

Table 11 provides a comparison between fatty acid patterns in modern egg, wild-type egg, fresh water fish, marine fish, marine inverterbrates, and marine mammals, with a subdivision for each class of fatty acids (i.e., SAFAs, MUFAs, PUFAs) between TFAs and LFAs based on their position on the lipid carrier. Simple mathematical computation estimates that a fatty acid evenly distributed in sn-1,2,3:TGs and sn-1,2:PLs of egg lipids would account for 63.33% TFAs and 36.67% LFAs (based on a TGs:PLs ratio of 16:6 in egg and neglecting the contribution of egg CE which represents less than 1% of total fatty acids) and a fatty acid evenly distributed between sn-1,2,3:TGs of river and marine oils would show up as 66.7% TFAs and 33.3% LFAs.

At this first level of analysis, the following two observations can be made: (i) the SAFAs content decrease from 31.8% in standard egg to 15.3% in marine mammals, and UFAs (MUFAs + PUFAs) compensates in one way or another and in a species-specific manner for this downward trend (i.e., river and ocean fish oils as well as marine mammal oils are richer in MUFAs whereas marine invertebrates have more PUFAs); and (ii) the T:L-distribution remains within the same predicted statistically balanced range for SAFAs (T:L = 60:40) and MUFAs (T:L = 70:30) whereas it favors LFAs for PUFAs (T:L = 50:50). The only conclusion that can be drawn at this level of analysis is that eggs have more SAFAs and therefore are expected to be more hypercholesterolemic than fish.

Table 11
Fatty Acid Distribution in Egg Lipids and Marine Oils

Fatty acids	*Modern egg*	*Wild-type egg*	*Fish freshwater*	*Fish marine*	*Invertebrates*	*Mammals*
SAFA	31.8	29.3	23.9	22.9	22.0	15.3
T.SAFA	64.4	58.6	66.6	53.6	76.8	53.4
L.SAFA	35.6	41.4	33.4	46.4	23.2	46.6
MUFA	46.3	41.9	59.0	61.4	47.8	61.5
T.MUFA	67.5	70.0	70.6	82.0	75.8	64.9
L.MUFA	32.5	30.0	29.4	18.0	24.2	35.1
PUFA	20.9	27.8	17.1	15.7	30.1	23.2
T.PUFA	50.6	57.1	55.6	54.5	43.9	79.4
L. PUFA	49.4	42.9	44.4	45.5	56.1	20.6
PUFA:SAFA	0.66	0.95	0.72	0.69	1.37	1.52
T.PUFA : T.SAFA	0.52	0.92	0.60	0.70	0.78	2.25
L.PUFA : L.SAFA	0.91	0.98	0.95	0.67	3.31	0.67

Abbr: T:FA: tissue-directed fatty acids, as released upon digestion of dietary lipids (sn-1/3:FFAs from TGs + sn-2:FFA from PLs); L:FA: liver-directed fatty acids (sn-2:MGs from TGs + sn-1:LPLs from LPs); SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Notes: Distributions between tissue- and liver-directed fatty acids for egg lipids and marine oils are averages computed, respectively, from analytical results obtained by structural analysis at Perugia University, Italy (unpublished proprietary information owned by Belovo SA; *see* Table 14) and from data published by Brockerhoff et al. (1968) and available in The Lipid Handbook (Gunstone, Harwood & Padley and from data published in ref. *26*).

Fish, fresh water were burbot, sheepshead, trout A, trout B and goldfish; fish, marine were herring, mackerel A, mackerel B, skate and cod; invertebrates were squid, periwinckle, lobster A, lobster B and scallop; mammals were harbour seal, harp seal and sei whale.

The second level of analysis focuses on the specific effect of these dietary lipids on blood cholesterolemia based on the type of message (P:S ratio) brought to peripheral tissues and liver. Within these species, the P:S ratio varies from 0.66 to 1.52 as a consequence of the decreased contribution of SAFAs and/or the increased contribution of PUFAs to the total fat content. The subdivision between T and L.P:S ratio's gives an additional indication about where (i.e., peripheral tissues and/or liver) egg lipids will act as messenger and trigger a transient cholesterolemic response. Table 11 shows that (i) modern eggs induce a transient hypercholesterolemic response through their delivery of dietary fatty acids with a low P:S ratio (0.52) to peripheral tissues and not so much (0.91) to the liver; (ii) wild-type eggs are a unique source of homeostatic dietary lipids and do not seem to send any lipidemic message whatsoever to either tissue (T.P:S = 0.92) or liver (L.P:S = 0.98) and are therefore not expected to contribute at all to transient blood cholesterol changes; (iii) fresh water fish lipids behave similarly to those of standard eggs, (iv) lipids from marine fish are definitely hypercholesterolemic because no matter their low SAFA content they still send a low P:S message to both peripheral tissues (T.P:S = 0.70) and liver (L.P:S = 0.67); (v) lipids from marine invertebrates exhibit a moderate hypercholesterolemic information to peripheral tissues (T.P:S = 0.78) largely compensated by a strong hypocholesterolemic message to the liver (L.P:S = 3.31); and (vi) lipids from marine mammals exhibit similar properties for the reverse

reasons—strong hypocholesterolemic information to the tissues (T.P:S = 2.25) only partially compensated by a moderate hypercholesterolemic message directed to the liver (L.P:S = 0.67). This second level of analysis allows us to draw a very different set of conclusions than those reached at the first level. It is clearly seen that the SAFA content of a food is not a sufficient indication for determining its transient influence on blood cholesterol. The literature has indeed sometimes identified fish oil as a hypercholesterolemic dietary supplement and clinical studies with modern and wild-type eggs have confirmed their dual influences on blood cholesterol *(27)*, in agreement with the conclusion from our structural analysis.

4.2.2. Triglyceridemic Effect

The third level of analysis is based on the known fact that ω-3 fatty acids (and to a lesser extent ω-6 fatty acids) have a strong depressing influence on postprandial secretion of very low-density lipoproteins (VLDL) by the liver. Therefore, the triglyceridemic effect of egg lipids and river/marine oils may be analyzed and compared with each other on the basis of their concentration in ω-3 fatty acids and on basis of the stereochemical position of these fatty acids on the two major lipid carriers (TGs and PLs) (Table 12): (i) modern eggs have little ω-3 (ω3-PUFA: 2.51%) and only a minor share of it (0.71%) is targeted to the liver, which probably results in relatively low effect on triglyceridemia; (ii) wild-type eggs have more ω-3 (ω3-PUFA: 13.84%) and a great deal of it (4.59%) is liver-oriented, allowing us to predict an overall hypotriglyceridemic effect for these eggs similar to that of fresh water fish (3.95%), marine fish (6.60%), and mammal oils (3.30%); and (iii) lipids from marine invertebrates appear to be particularly efficient in this respect because 16% ω-3 fatty acids in these oils are destined to end up in the liver and to repress enzymes involved in the expression of fatty acid synthase genes. Again, earlier published results *(27,28)* seem to come in full support of these predictions from structure–function analysis. As blood triglycerides have recently been recognized as a determinant factor in atherogenicity (to promote production of small dense LDL particles), it is interesting to note that wild-type eggs are just as efficient as fish oils vs hypertriglyceridemia in humans.

4.2.3. Tissue Incorporation

The fourth level of analysis focuses on the efficiency of tissue incorporation for ω-3 fatty acids from these different dietary sources (Table 12). As seen, wild-type eggs remain an excellent source of ω-3 fatty acids to tissue (9.25%) even if ALA accounts for more than 80% (7.56/9.25) of them. The fact that this EFA is targeted to peripheral tissue rather than to the liver protects it against immediate β-oxidation and gives the body the opportunity to elongate it to ω3-LCPs, mainly EPA *(29)* where and when there is a need for it. In fact, this specific arrangement of EFAs and LCPs in eggs makes wild-type eggs a unique source of ω-3 fatty acids to body tissues and, at the same time, a barrier against over-vitaminosis. In addition, the yield of transfer of ω3-LCPs from these dietary lipids to tissue cell membranes (ω3-T.LCPs) varies within this group of foods in the sense that, on a similar weight basis, eggs (both modern and wild-type) and marine mammals ω3-LCPs are ±85/50 = ±1.7 time more efficiently transferred to tissue through digestion than ω3-LCPs from river and marine fishes for which 50% of ω3-LCPs are found at the sn-2 position of TGs, with a potential risk of being catabolized in the liver

Table 12
PUFAs Distribution in Egg Lipids and Marine Oils

PUFAs	*Modern egg*	*Wild-type egg*	*Fish freshwater*	*Fish marine*	*Invertebrates*	*Mammals*
ω3-PUFA	2.51	13.84	7.9	13.8	27.2	20.9
ω3-T.PUFA	1.80 (71.7)	9.25 (66.8)	(3.95) 50.0	7.20 (52.2)	11.2 (41.0)	17.6 (84.4)
ω3-L.PUFA	0.71 (28.3)	4.59 (33.2)	(3.95) 50.0	6.60 (47.8)	16.0 (59.0)	3.30 (15.6)
ω3-EFA	0.9	11.8	–	–	–	–
ω3-T.EFA	0.37 (41.2)	7.56 (64.1)	–	–	–	–
ω3-L.EFA	0.53 (58.8)	4.44 (35.9)	–	–	–	–
ω3-LCP	1.61	2.04	7.9	13.8	27.2	20.9
ω3-T.LCP	1.43 (89.1)	1.69 (82.7)	(3.95) 50.0	7.20 (52.2)	11.2 (41.0)	17.6 (84.4)
ω3-L.LCP	0.18 (10.9)	0.35 (17.3)	(3.95) 50.0	6.60 (47.8)	16.0 (59.0)	3.30 (15.6)
ω6-PUFA	18.3	14.0	9.2	1.9	2.9	2.3
ω6-T.PUFA	8.74 (47.8)	6.65 (47.5)	5.55 (60.3)	1.37 (72.0)	2.05 (70.7)	0.80 (35.0)
ω6-L.PUFA	9.56 (52.2)	7.35 (52.5)	3.65 (39.7)	0.53 (28.0)	0.85 (29.3)	1.50 (65.0)
ω6-EFA	15.6	13.4	9.2	1.9	2.9	2.3
ω6-T.EFA	6.27 (40.2)	6.14 (45.8)	5.55 (60.3)	1.37 (72.0)	2.05 (70.7)	0.80 (35.0)
ω6-L.EFA	9.33 (59.8)	7.26 (54.2)	3.65 (39.7)	0.53 (28.0)	0.85 (29.3)	1.50 (65.0)
ω6-LCP	2.7	0.6	–	–	–	–
ω6-T.LCP	2.47 (91.5)	0.51 (85.8)	–	–	–	–
ω6-L.LCP	0.23 (8.50)	0.09 (14.2)	–	–	–	–
ω6:ω3	7.29	1.01	1.16	0.14	0.11	0.11

Abbr: EFA: essential fatty acids (ω3:EFA or ALA or alpha-linolenic acid; ω6:EFA or LA or linoleic acid); LCP: long chain polyunsaturated fatty acids; other abbreviations as in Table 11.

Note: Distributions between tissue- and liver-directed fatty acids for egg lipids and marine oils are averages computed from data sourced as explained under Table 11. Numbers within brackets are in %.

or diluted in adipose tissues. Another study *(30)* demonstrated the efficiency of transfer of ω-3 fatty acids from wild-type eggs to human body tissue through the influence of the consumption of such eggs on the fatty acid composition of breast milk. In that study, it was shown that the efficiency of transfer form egg to milk through breast tissue was such that 5 wild-type eggs in 1 wk (or less than 100 mg ω3-LCPs/d) was sufficient to triple (from 0.4 to 1.2%) the amount of ω3-LCPs in milk fat or three times the minimum amount (20 mg/150 mL milk) recommended by the FAO/WHO for infant nutrition.

4.3. Essential Difference Between Land-Based and River/Marine Essential Lipids

As seen in Table 12, river and marine animals are essentially deprived of ω3-EFAs and ω6-LCPs whereas they are well represented in the lipids of hen eggs and land-based animals in general. In addition, there seems to exist an inverse relationship between the amount of essential fatty acids in land-based and river/marine animals; indeed, a much higher level (11.8%) of ω3-EFAs in the wild-type egg than in the modern egg (0.9%) does not translate to a substantially higher level of ω3-LCP in the former rather than the latter (2.04% vs 1.61%), but rather in a sharp reduction (–77.5%) of ω6-LCPs in the former compared with the latter (0.6% vs 2.7%). This observation fits with current scientific consensus that ALA is not a good source of ω3-LCPs in land-based animals (and in humans) and that it acts indirectly on tissue composition through inhibition of the fatty acid metabolic pathway leading to elongation of ω6-EFA to ω6-LCPs.

Omega-3 LCPs are present in all species, but sea animal lipids are much richer in those fatty acids (Σω3:T:LCPs for direct tissue incorporation range from ± 1.5% in egg—standard or Columbus®—to 17.64% in marine mammals); Omega-6 EFA (linoleic acid) is present in all species, but eggs do contain more of this EFA than sea animals; the ω6:ω3 ratio in Columbus® egg and fresh water fish are similar but Columbus® egg has the land based characteristic fatty acids (ω3-EFAs and ω6-LCPs) which river fishes miss; marine oils have extremely low ω6:ω3 ratios (± 0.1) because of their relatively low level of ω6-LCP and their high loads of ω3-LCPs.

4.4. Desaturation Index

Table 13 compares the desaturation indexes (the average number of double bonds/fatty acid), in the various lipid sources. As seen, standard eggs represent a monounsaturated lipid source (DI = ±1) and the DI increases as expected with an increasing contribution of EFAs and LCPs in the other species. DIs also confirm that marine mammals are excellent sources of PUFA for tissue incorporation (DI: 1.91 for sn-1/3:TG) whereas lipids from marine invertebrates are outstanding sources of PUFA to the liver (DI: 2.83 for sn-2:TG). In eggs, the following observations are worth noting:

- The DI in Columbus® egg is slightly higher (1.16) than that in standard egg (0.98) resulting from an increased level of double bonds (ALA) in the TG fraction of these eggs.
- The trend is reversed in the PL fractions resulting from a reduction (from 1.21 to 0.96) of the DI in the PC fraction of Columbus® egg, the DI in the PE fraction remaining unchanged (1.69 vs 1.62); the PE fraction in egg is known to be the major source of LCPs to the brain of the chick embryo and, as such, has a crucial role to play in embryogenesis *(31)*.

Table 13
Desaturation Index in Egg Lipids and Marine Oils

Desaturation index	*Modern egg*	*Wild-type egg*	*Fish freshwater*	*Fish marine*	*Invertebrates*	*Mammals*
DI (global)	0.98	1.16	–	–	–	–
TG	0.89	1.18	1.13	1.29	1.87	1.61
PL	1.30	1.08	–	–	–	–
PC	1.21	0.96	–	–	–	–
PE	1.69	1.62	–	–	–	–
sn-1/3:TG	0.68	1.01	1.05	1.22	1.40	1.91
sn-2:PL	2.33	2.01	–	–	–	–
sn-2:PC	2.32	1.81	–	–	–	–
sn-2:PE	2.36	2.93	–	–	–	–
sn-2:TG	1.33	1.52	1.31	1.45	2.83	1.04
sn-1:PL	0.21	0.21	–	–	–	–
sn-1:PC	0.18	0.17	–	–	–	–
sn-1:PE	0.33	0.39	–	–	–	–

Note: Desaturation Indexes (DIs) or "average number of double bonds per fatty acid" in TG fractions of egg lipids and marine oils and in PL fractions of egg lipids are averages computed from data sourced as explained under Table 11.

- Structural analyses show that it is the sn-2 fatty acids of PC (sn-2:PC) which is the most affected (DI: 2.32 in standard egg compared to 1.81 in Columbus® egg).
- The fatty acid at the sn-2 position of both TG and PL fractions (including PC and PE) of eggs exhibit a much higher DI than the fatty acids at position sn-1/3 of TGs and sn-1 of PLs.

5. CONCLUSIONS

5.1. Eggs vs River/Sea Lipids

When comparing egg lipids and river/sea animal oil composition, one realizes that ω-3 EFAs (mainly ALA) and ω-6 LCPs (mainly AA) are characteristics of land based animals and that these two fatty acids are in competition for presence in dietary lipids from animal origin; in addition, their respective concentration in animal food seems to depend solely on the share of ALA in the animals' dietary lipids.

It is also noteworthy that the wild-type eggs have all the characteristics of a hypolipidemic food because the sum of all LFA in the egg accounts for (11.8% ω3-EFA × 35.9%) + (2.04% ω3-LCP × 17.3%) + (13.4% ω6-EFA × 54.2%) + (0.6% ω6-LCP × 14.2%) = 12% and is only surpassed by marine invertebrates (LFA: 16.9%). On the other hand, the wild-type egg is the next best carrier for PUFAs to tissues with a TFA of 15.9% just below that of marine invertebrates (T.FA: 18.44%). Finally, it is seen that the tissue bioavailability of ω3-LCP from eggs stands high at between 82.8 and 88.8%—a value similar to that observed for marine mammals (84.4%) and much higher than that observed for fresh and sea water fish and invertebrates (50.0, 52.2 and 41.0%).

5.2. The Null Relationship Between Dietary CHL and CHD

The question that remains is whether egg cholesterol should remain an issue for human nutrition. Certainly, there is no current indication on the minimum cholesterol daily intake which would be beneficial to humans. There are two main reasons for this: (i) the human body produces enough cholesterol to fulfil its requirement, and (ii) dietary cholesterol has been singled out for more than 40 yr as the major culprit in cardiovascular disease (CVD) and coronary heart disease (CHD). However, recent reviews *(32,33)* of epidemiological and clinical studies carried out in the last quarter of the century have demonstrated the null relationship between dietary CHL and CHD. Certainly, dietary CHL does increase blood cholesterol as initially identified by Keys and Hegsted *(21,22)*, but: (i) the extent of the increase depends on individual physiological and genetic factors and, in healthy individuals, it does not influence the LDL:HDL atherogenic ratio and is thus of no influence whatsoever on CHD; and (ii) the short-term increase in blood cholesterol usually triggers metabolic change which translates into a return to normal blood cholesterol levels in the long term; an observation confirming epidemiological data indicating that the intake of dietary cholesterol is not related to CHD in the general population.

Reanalyzing epidemiological studies for confounding factors, McNamara has provided evidence that dietary SAFAs were the leading factor in CHD, but Okuyama has raised the point that high SAFA is a surrogate indicator for high intake of animal fat with high ω6:ω3 ratio and that in fact it is this single ratio that seems to be the best candidate for atherogenic factor in the standard modern human diet.

In fact, currently available scientific reviews reveal that healthy functional dietary cholesterol would help extend human lifetime provided it is associated with a balanced, wild-type, EFA ratio. Therefore, in a search towards the ideal functional food for human, one may end up with the wild-type egg.

6. FUTURE RESEARCH

In the future, it would be interesting to rely on similar fatty acid reference patterns for infant and all other-age feeding as those available for EAAs (i.e., breast milk composition and nitrogen balance studies). There are some restricting factors inherent to this approach for dietary lipids: (i) human tissues and breast milk fatty acid compositions are sensitive to dietary fats and there is no real unique standard available; perhaps, the "gold standard" could be approached through feeding cohorts of humans a wild-type diet and analyzing its influence on body tissue and breast milk composition; (ii) there is no single marker (i.e., nitrogen for amino acids) to follow body excretion of fatty acid metabolites, and therefore fat balance studies must also take into account that most excesses in fat intake will be transferred to fat depots, the major reservoir for energy in the body, therefore requiring accurate measurement of body fat variation and metabolism in balance studies; and (iii) cholesterol and long chain polyunsaturated fatty acids (LCPs) are only found in animal tissues and, for humans in the industrialized world, animal products contribute more than 60% of the total lipids, 70% of the saturated fats, and 100% of the cholesterol in the diet.

As final conclusion, the following facts about eggs seem to gain credibility: (i) egg is a light food, contains no carbohydrates, and is hypo-insulinolemic; (ii) egg is balanced

Table 14
Structural Fatty Acid Analysis in Wild-Type Egg (W) and EU-Modern Egg (M)

	Wild-type egg (W)										*EU-Modern egg (M)*									
	TG				*PC*			*PE*			*TG*				*PC*			*PE*		
FAME	*Tot.*	*sn-1*	*sn-2*	*sn-3*	*Tot.*	*sn-1*	*sn-2*	*Tot.*	*sn-1*	*sn-2*	*Tot.*	*sn-1*	*sn-2*	*sn-3*	*Tot.*	*sn-1*	*sn-2*	*Tot.*	*sn-1*	*sn-2*
C14:0	0.3	0.6	0.1	0.2	0.2	0.5	0.1	0.1	–	0.1	0.6	0.9	0.2	0.7	0.2	0.3	0.1	0.1	–	0.1
C15:0	0.1	0.1	–	0.2	0.2	0.3	0.1	0.1	–	0.1	0.1	0.2	–	0.1	0.1	0.2	–	0.1	0.2	–
C16:0	19.2	52.9	2.1	2.6	27.9	54.2	1.7	13.5	28.5	0.6	24.0	64.3	2.4	5.3	22.4	48.5	0.8	15.0	33.0	0.7
C17:0	0.2	0.5	–	0.1	0.2	0.4	–	0.3	0.8	–	0.3	0.8	0.1	–	0.3	0.7	–	0.4	0.7	–
C18:0	4.8	5.5	1.4	7.5	13.0	28.0	0.4	24.0	55.0	0.3	4.8	4.9	1.8	7.7	15.7	31.5	0.6	21.7	39.3	0.4
C14:1ω5	0.1	–	–	0.3	–	–	–	–	–	–	0.1	0.1	–	0.2	–	–	–	–	–	–
C16:1ω7/9	4.1	5.7	1.4	5.2	1.7	2.2	1.0	0.5	0.4	0.5	4.2	6.1	1.6	4.9	1.2	1.8	0.4	0.5	0.5	0.5
C17:1ω8?	0.2	0.1	–	0.5	0.1	–	0.2	–	–	0.1	0.3	0.2	0.2	0.5	0.1	0.1	0.1	0.1	–	0.1
C18:1ω7/9	37.8	13.7	37.4	62.3	27.8	8.0	47.4	19.9	6.2	33.3	48.7	19.5	54.0	72.6	22.7	11.4	31.4	17.6	12.2	25.3
C20:1ω9	0.1	–	0.1	0.2	0.1	0.2	0.0	0.1	–	0.3	0.1	–	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.2
C18:2ω6	14.7	2.6	35.7	5.8	16.5	3.1	26.9	11.1	2.2	17.8	15.0	1.6	36.7	6.7	15.1	4.4	22.3	12.2	6.0	18.5
C20:2ω6	0.1	–	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.1	–	0.2	0.1	0.2	0.1	0.5	0.3	0.2	0.5
C20:4ω6	0.1	–	0.3	–	1.8	0.1	3.8	6.2	0.5	10.0	0.3	–	0.6	0.3	11.4	–	21.4	16.4	5.8	26.7
C22:4ω6	–	–	–	–	–	–	0.1	0.1	–	0.2	–	–	–	–	0.6	–	1.6	1.1	0.1	1.5
C22:5ω6	–	–	–	–	–	–	0.2	0.1	–	0.1	–	–	–	–	2.0	–	4.7	3.1	0.1	5.9
C18:3ω3	17.2	18.0	19.3	14.3	3.4	2.1	3.6	2.7	1.8	2.9	0.8	1.2	1.2	–	0.2	0.1	0.3	0.2	–	0.6
C18:4ω3	0.1	0.2	–	0.1	0.1	0.1	0.2	0.1	0.3	0.1	0.3	0.1	0.2	0.6	0.2	0.1	0.3	0.2	0.1	0.3
C20:3ω3	0.2	0.1	0.3	0.2	0.2	0.1	0.3	0.2	0.1	0.2	–	–	–	–	–	–	–	–	–	–
C20:5ω3	0.1	–	0.2	0.1	0.3	–	0.8	2.6	0.3	3.8	–	–	–	–	–	–	0.2	–	–	0.2
C22:5ω3	0.3	–	0.8	0.1	0.7	0.1	1.3	2.1	2.3	2.7	0.1	–	0.3	–	0.6	–	1.3	0.9	0.2	1.3
C22:6ω3	0.3	–	0.7	0.2	5.7	0.5	11.7	16.1	1.5	26.7	0.2	0.1	0.4	0.1	6.9	0.6	13.8	9.9	1.5	17.2

Abbr: (–) indicates not detectable amounts or less than 0.1%.

in protein an d lipids and fits the human requirement for tissue and organ building and repair (P:L = 1:2); (iii) egg protein mirrors human tissue protein composition (only Leu is slightly deficient for infants when compared with breast milk); (iv) wild-type egg lipids deliver EFAs in excellent agreement with the human requirement, resulting in improved lipidemia (better cholesterol distribution and reduction in triglycerides) and tissue/breast milk composition; and (v) new sets of recent data in the literature tends to favor dietary cholesterol because long-term prospective follow-up studies performed in Japan and Western countries concluded individuals with higher plasma cholesterol survive longer, possibly resulting from decreased cancer mortality, decreased mortality from infectious diseases, and/or decreased apoplexy *(33)*.

It thus appears that the wild-type egg is a nutritional "ideal" or "optimum" in human nutrition, thanks to its perfectly balanced amino acid *and* fatty acid (lipid) compositions. In fact, it provides all essential elements to tissue-repair in human and no carbohydrates. From this perspective, it is best described as the "zero-calorie body rejuvenating food" or "Magic Bullet" *(34)*.

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9 The Essentiality of Eicosapentaenoic Acid in Breast Milk During Human Lactation

Basant K. Puri and Jonathan P. Stannard

Abstract

The case is described of a European woman in her thirties whose diet contained very little n-3 long-chain polyunsaturated fatty acids apart from an intake of one docosahexaenoic acid-enriched egg daily over the previous 6 yr. This enrichment process was carried out by feeding hens a diet close to that of their wild ancestor, the Jungle Fowl, by including a wider range of seeds and green vegetation than is normally the case in modern farming practice. During this period the subject had two full-term normal pregnancies. Analyses of her breast milk during the postnatal periods have consistently shown that her milk was richer in both eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) than average European human milk from lactating women. This study demonstrates that it is ideal for lactating women to consume a dietary source of long-chain polyunsaturated fatty acids from which they can provide both eicosapentaenoic acid and docosahexaenoic acid for their baby. The study also indicates that current average Western human milk is considerably lower in eicosapentaenoic acid than is optimal and we would recommend further study in this area to define optimal fatty acid levels for lactating women based on a balanced source of dietary lipids, rather than one based on a diet lacking in these fatty acids which we believe produces inadequate levels of eicosapentaenoic acid in human milk.

Key Words: Eicosapentaenoic acid; breast milk; lactation; bioavailability.

1. LONG-CHAIN POLYUNSATURATED FATTY ACID IN HUMAN BREAST MILK

1.1. Biosynthesis

It is generally agreed that the biosynthetic pathway of n-3 long-chain polyunsaturated fatty acids from eicosapentaenoic acid includes the conversion of eicosapentaenoic acid (C20:5n-3) to docosapentaenoic acid (C22:5n-3) via an elongase reaction. However, the next stage, of conversion of C22:5n-3 into C22:6n-3, has been the subject of controversy. Rather than following direct Δ-4-desaturation of C22:5n-3 into C22:6n-3, in humans it has been suggested that the following steps are involved: the conversion of docosapentaenoic acid (C22:5n-3) to tetracosapentaenoic acid (C24:5n-3) via an elongase reaction; the conversion of tetracosapentaenoic acid (C24:5n-3) to tetrahexaenoic acid (C24:6n-3) via Δ-6-desaturase; and finally, the "retroconversion" of tetrahexaenoic acid (C24:6n-3) to docosahexaenoic acid (C22:6n-3) via β-oxidation. The recent demonstration that human cells from patients with deficiencies of either acyl-CoA oxidase

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or D-bifunctional protein, the first two enzymes of the peroxisomal straight-chain fatty acid β-oxidation pathway, show impaired conversion of either [1-14C]18:3n-3 or [1-14C]22:5n-3 to docosahexaenoic acid, and the finding that acyl-CoA oxidase deficient cells exhibit a two- to five-fold accumulation of radiolabeled tetrahexaenoic acid compared with control cells, are consistent with the second proposed pathway from eicosapentaenoic acid to docosahexaenoic acid *(1)*.

1.2. Function

Whereas docosahexaenoic acid has important structural roles in the membranes of cells and intracellular organelles, it has been suggested that eicosapentaenoic acid may also be of great importance owing to its various functional roles, including as a precursor to certain eicosanoids *(2–4)*. Both eicosapentaenoic acid and docosahexaenoic acid are important components of normal human milk in the lactating postnatal female *(5)*; for example, infants fed with infant formula preparations enriched with eicosapentaenoic acid and docosahexaenoic acid have better visual attention and memory recognition than those fed with ordinary formula preparations *(6)*.

2. DIETARY SUPPLEMENTATION

2.1. α-Linolenic Acid

Supplementing the diet of lactating women with flaxseed oil, which is a relatively good source of the essential n-3 fatty acid α-linolenic acid (C18:3n-3), has been found to increase levels of eicosapentaenoic acid in plasma and breast milk, but not the levels of docosahexaenoic acid in plasma, breast milk or erythrocyte membranes *(7)*. The reason for the poor conversion of α-linolenic acid or of the derived eicosapentaenoic acid into docosahexaenoic acid in lactating women is not known. However, it is known that increasing the level of α-linolenic acid does have positive benefits in respect of cardiovascular disease. A recent review by Lanzmann-Petithory *(8)* noted that: "It is only when the diet was enriched in n-3 FA, especially alphalinolenic acid (ALA) that cardiac death was reduced. Studies in animals as well as in vitro on myocytes in culture, have shown that ALA was preventing ventricular fibrillation, the chief mechanism of cardiac death. Furthermore, studies in rats have observed that among n-3 FA, ALA, the precursor of the n-3 family, may be more efficient to prevent ventricular fibrillation than eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In addition it was demonstrated that ALA was the main FA lowering platelet aggregation, an important step in thrombosis, i.e., non fatal myocardial infarction and stroke."

Because both eicosapentaenoic acid and docosahexaenoic acid are required in human breast milk for healthy infant development, one possible way of ensuring high levels of these long-chain fatty acids in breast milk might be to supplement the diet of lactating women with docosahexaenoic acid. A suitable vector for this fatty acid is the docosahexaenoic acid-rich egg. In view of the above findings, it would also be good if such eggs were rich in alpha-linolenic acid.

2.2. Columbus Eggs

The dietary α-linolenic acid- and docosahexaenoic acid-rich egg used in this study was the Columbus Egg (Deans Foods, UK and Belovo SA, Belgium). The production

Table 1
A Comparison of the Fat Contents of Typical Eggs With Columbus Eggs

	Standard egg	*Columbus Egg*
α-linolenic acid	30 mg	550 mg
ω-3 long-chain polyunsaturated fatty acids	45 mg	110 mg
ω-6:omega-3	10:1	1:1
SAFA	32.5%	30.0%
MUFA	45.0%	40.0%
PUFA	22.5%	30.0%

of these eggs entails feeding hens a diet similar to that eaten by their ancestors in the wild. This diet is composed mainly of seeds and green leaves. Whereas the percentage of fatty acids in a standard egg that are polyunsaturated, n-6 polyunsaturated and n-3 polyunsaturated are approx 13.6, 13.3, and 0.3, respectively, the corresponding percentages for a Columbus Egg are 29.3, 14.7, and 14.6. Thus, not only does a Columbus Egg have twice the level of polyunsaturated fats that are found in a standard egg, the ratio of n-6 to n-3 long-chain polyunsaturated fatty acids is one, as was generally the case in the (Western) human diet before the middle of the nineteenth century *(9)*. Almost the whole of the n-3 long-chain polyunsaturated fatty acid fraction in a Columbus Egg is in the form of phospholipid docosahexaenoic acid, with the docosahexaenoic acid being attached at the sn-2 position and so readily releasable from the phospholipid glycerol backbone in the jejunum by the action of pancreatic 2-phospholipase. Table 1 compares the fat content of typical eggs with Columbus Eggs.

3. SERIAL LONGITUDINAL HUMAN STUDY

3.1. Methods

In 1998, the subject, a healthy 30-yr-old nonsmoking female, started consuming one Columbus Egg daily, and has continued to do so ever since. This constituted her main intake of n-3 long-chain polyunsaturated fatty acids. She did not take fatty acid supplementation and dislikes fish, with an intake of oily fish limited to a maximum of one small helping no more frequently than once every 2 mo. In October 2001, the subject gave birth to a healthy daughter after a pregnancy lasting 39.5 wk. In February 2002, the first breast milk sample from her was taken for analysis. She became pregnant in 2002 and gave birth again in June 2003 to a healthy son, again after a pregnancy lasting 39.5 wk. A second breast milk sample was taken in September 2003. A third and fourth sample were taken in March 2004 and June 2004, respectively. The subject was breast-feeding during the whole period covered by the breast milk samplings. Fatty acid methyl esters were analyzed with gas-liquid chromatography.

3.2. Results

The n-3 long-chain polyunsaturated fatty acid results for the n-3 long-chain fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) are shown in Figs. 1–3.

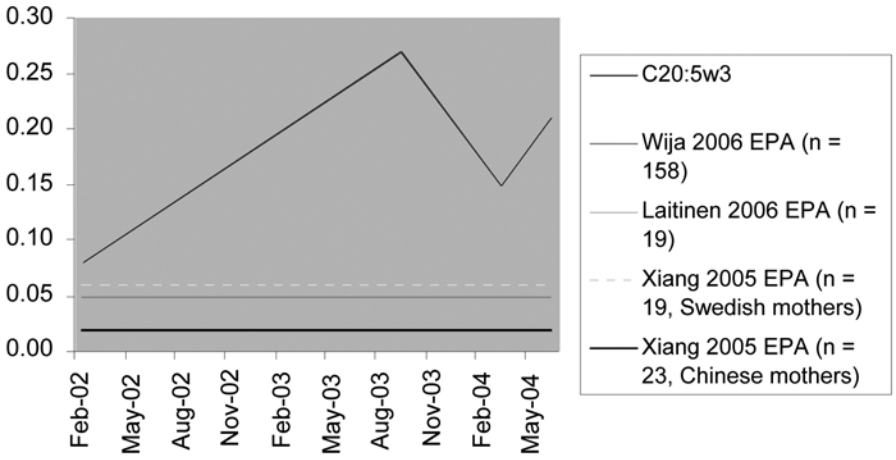

Fig. 1. Subject's EPA in human milk curve against recent EU averages.

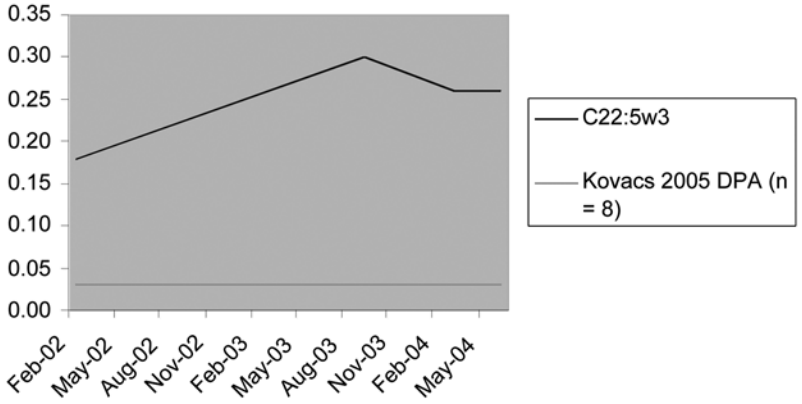

Fig. 2. Subject's DPA in human milk curve against recent EU averages.

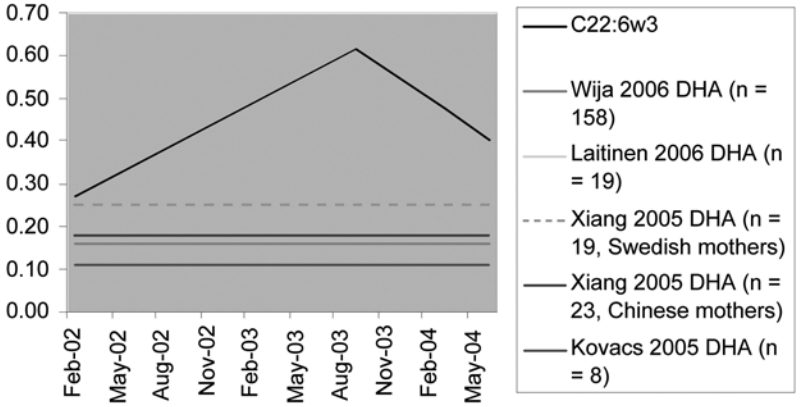

Fig. 3. Subject's DHA in human milk curve against recent EU averages.

By comparison, the levels of EPA, DPA, and DHA found in human breast milk in recent European studies are also shown in Figs. 1–3. These are derived from studies by Wijga et al., a Dutch study of 107 nonallergic mothers *(10)*, Kovács et al., a Hungarian study of 10 full-term controls *(11)*, Laitinen et al., a Finnish study of 19 normal control subjects *(12)*, and Xiang et al., a comparison of 19 Swedish mothers with 23 Chinese mothers *(13)*.

4. DISCUSSION

The results clearly show that consumption of one Columbus Egg daily was sufficient to lead to breast-milk levels of EPA, DPA, and DHA which were far higher than those measured in recent European studies. Columbus Eggs therefore provide a highly bioavailable form of ω-3 fatty acids; this may be because the ω-3 and ω-6 fatty acids in this food are balanced, being closer to the ratio of approx 1:1, which was typically part of our ancestors' diets.

The fact that EPA appeared in the subject's milk in high concentrations compared with average European milk strongly suggests that formula milk is seriously short of a clearly vitally important long-chain polyunsaturated fatty acid. Formula preparations have been based, in part, on the composition of Western human milk. However, because the average diet of mothers in the West is compromised with respect to fatty acids, their milk tends to be low in EPA.

The high levels of DHA in the subject's milk were clearly derived from her diet; high levels of DPA indicate a conversion aided by matched ratios of linolenic acid to α-linolenic acid.

Although women can lactate for as long as they wish we could not find a single study examining fatty acids at this length of breastfeeding. However, the composition of the milk indicates that there is an inclination for the body to feed the growing baby for a length of time considerably longer than that at which babies are currently weaned (usually well before 1 yr, in the West). Lauritzen et al. *(14)* have shown brain accretion of DHA at an exponential rate for 2 yr postnatally, at which point it levels off but continues in requirement into the teens.

Finally, we hypothesize that the cause of the plethora of neurodevelopmental disorders in childhood and beyond may be a response to the poor consumption of fatty acids such as EPA during the last two generations. If this is correct, then it may be possible to make an important impact on the level of these disorders if we return to a diet which includes balanced fatty acids, and if we feed babies with human milk (containing adequate levels of EPA) for a longer period or formula feed suitably enriched with a wider range of fatty acids (including EPA).

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10 The Natural Fatty Acid Compositions of Eggs of Wild Birds and the Consequences of Domestication

Peter F. Surai and Brian K. Speake

Abstract

The fatty acid profiles of yolk lipids vary greatly among avian species in the wild. This results partly from the major differences in the pattern of polyunsaturated fatty acids (PUFAs) provided by different dietary modes. For example, granivorous species that obtain linoleic acid (18:2ω-6) from dietary seed oils incorporate high proportions of 18:2ω-6 into their yolk lipids. By contrast, yolks of herbivorous birds are rich in α-linolenic acid (18:3ω-3), derived from the lipids of grasses and leaves. Arachidonic acid (20:4ω-6) is the characteristic yolk PUFA of carnivorous birds, because this fatty acid is well represented in the tissue lipids of their prey. Typically, docosahexaenoic acid (22:6ω-3) and other ω-3 HUFA derived from fish lipids predominate in the yolks of piscivorous birds. Yolk fatty acid composition is also influenced by genetic factors relating to aspects of maternal metabolism that determine the selective incorporation of particular PUFAs into the yolk. Ultimately, the yolk fatty acid profile results from the interplay between diet and phylogeny. The effects of phylogeny are illustrated by, for example, the similar fatty acid compositions of the yolks of several species of ducks with different dietary preferences. Most species of birds in the wild lay eggs that have ω-6:ω-3 ratios close to unity. This contrasts with eggs of the domestic chicken where this ratio is greater than 10:1. The fatty acid profiles of eggs laid by pheasants, geese, ostriches, partridges, ducks, and kestrels in captivity are markedly different from those of the same species in the wild. In particular, the yolks of the captive birds are relatively deficient in 18:3ω-3, 22:6ω-3 or both, and have much higher ω-6:ω-3 ratios, in comparison with the values for the yolks of their free-living counterparts. Considering all avian species for which data is available, the mean yolk ω-6:ω-3 ratio in the wild = 1.52 ± 0.99 (mean ± SD; n = 23 species). The mean value of this ratio for species in captivity = 15.8 ± 9.5 (n = 9 species). On average, therefore, the yolk ω-6:ω-3 ratio is some 10 times greater in captivity than in the wild. In this light, the fortification of table eggs with ω-3 PUFAs to enhance human health can simply be seen as a return to the natural situation.

Key Words: Fatty acids; eggs; wild birds; and ω-3 PUFAs.

1. INTRODUCTION

1.1. Yolk Fatty Acids and the Nutritional Needs of the Avian Embryo

Although birds' eggs have been a nutritious food source for humans since prehistoric times, it is important to remember that egg composition has evolved primarily to meet the nutritional needs of the avian embryo. For example, the fatty acid profile of the yolk lipids must be suited to the specific requirements of the differentiating embryonic tissues. Most notably, the cell membrane phospholipids of the brain, retina, and skeletal muscle

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of the embryo must acquire high proportions of docosahexaenoic acid (DHA) (22:6ω-3) at defined stages of development *(1,2)*. This long-chain highly unsaturated fatty acid (HUFA) of the ω-3 (ω-3) series is believed to be vital for the functional development of these "excitable" tissues because of the unique biophysical properties that it imparts to cell membranes *(3)*. Similarly, the phospholipids of the heart, muscle, liver, and brain of the avian embryo become highly enriched with the ω-6 HUFA, arachidonic acid (20:4ω-6), at the appropriate developmental stages *(1,4)*. The importance of 20:4ω-6 in tissue development relates to its roles in signal transduction and eicosanoid synthesis *(1,4)*. In principle, the differentiation of each cell type during avian development is accompanied by the expression of a characteristic phospholipid fatty acid composition appropriate to the function of that tissue.

The selective distribution of the various fatty acids among the embryonic tissues is largely achieved by preferential transport, uptake and incorporation mechanisms *(1,5,6)*. Ultimately, however, the ability to achieve the desired cell-specific membrane fatty acid profile is dependent on an adequate supply of the appropriate fatty acids from the yolk. Thus, the yolk lipids must contain sufficient amounts of 22:6ω-3 and 20:4ω-6 to support tissue development. Alternatively, these HUFAs may in principle be synthesized in the embryo by desaturation/elongation of their C18 precursors, namely linoleic (18:2ω-6) and α-linolenic (18:3ω-3) acids *(1,7,8)*. In this case, the yolk lipids would need to contain adequate amounts of 18:2ω-6 and 18:3ω-3 for this purpose. The desaturation/elongation pathway is known to be expressed in the yolk sac membrane and the liver of the chicken embryo *(1,7,8)* but the quantitative importance of this pathway is unclear. In fact, current evidence suggests that most of the 22:6ω-3 that accumulates in the embryonic tissues of the chicken is derived from the "preformed" 22:6ω-3 in the yolk lipid, as opposed to biosynthesis from 18:3ω-3 *(9)*. However, the desaturation/elongation pathway may assume greater importance in embryos of some other avian species, particularly in those species whose yolks are naturally rich in C18 essential fatty acids (EFA) but are poor in HUFA.

In summary, the PUFA profile of egg yolks of avian species must meet certain minimum requirements. The amounts of HUFA (or their C18 precursors) in the yolk lipids, and the ω-6:ω-3 balance, must be appropriate for the needs of embryonic tissue development. Although yolk fatty acid compositions vary widely among free-living avian species, all reported examples contain some 22:6ω-3 and 20:4ω-6, and many display high proportions of the C18 EFA.

Although we have emphasized the importance of HUFA and EFA in avian embryonic development, it should be pointed out that the monounsaturated fatty acid, oleic acid (18:1ω-9) is quantitatively the most abundant fatty acyl component of yolk lipid in eggs of almost all species that have been studied. Yolk lipids are also relatively rich in saturated fatty acids, particularly palmitic acid (16:0). Apart from their role in cell membrane phospholipid formation, a major fate of yolk fatty acids is to be oxidized to provide the energy for development *(1)*. Typically, about 50% of total yolk lipid is metabolized for energy by the β-oxidation pathway during the embryonic period *(9)*. Interestingly, in the chicken embryo, 22:6ω-3 and 20:4ω-6 are to some extent diverted away from the β-oxidation pathway and are preferentially incorporated into tissue phopholipids *(9)*. A high proportion of 18:1ω-9 seems to be vital for successful embryogenesis because a reduction in the proportion of this fatty acid in egg lipid (caused by

inhibition of δ9 desaturase activity in the liver of the parent hen) can seriously impair development *(10)*.

1.2. Interspecies Variation in Yolk Fatty Acid Profiles

Yolk fatty acid compositions, particularly the PUFA profiles, vary dramatically among avian species in the wild. For example, the proportion of 22:6ω-3 in yolk lipid of different species varies over a 15-fold range, based on the data currently available, whereas the proportion of 18:3ω-3 varies by a factor of at least 200. Many species display a highly characteristic egg fatty acid composition, providing a clear distinction from eggs of other species. However, egg composition of some species can vary according to geographical location. For example, the proportion of 18:3ω-3 was found to be 8 times greater in eggs of the Canada goose (*Branta canadensis*) collected in southern England compared with those of the same species collected in Saskatchewan *(11*, and unpublished data). This difference may be due to diet since the English population of *B. canadensis* consumed mainly grass (a source of 18:3ω-3) whereas the Canadian population had greater access to grain (a source of 18:2ω-6) from agricultural land. Other sources of variation within a species, such as the changing availability of different food items, the sequence of eggs in a clutch, and the sequence of clutches throughout the breeding season, have yet to be investigated. Nevertheless, eggs of many species are characterized by a highly distinctive fatty acid "signature." In some cases, this may be unique to that species. In other instances, the "signature" may be more broadly characteristic of a higher taxonomic group such as family or order or, alternatively, may encompass species that share a particular dietary mode.

Despite the tremendous interspecies variation in yolk fatty acid profiles, the tissue-specific phospholipid fatty acid compositions of the newly hatched chicks of the various species tend to be very similar. For example, the proportions of 22:6ω-3 in brain phospholipid of 6 avian species at hatch were almost identical, even though the proportion of 22:6ω-3 in their yolk lipids varied over a sixfold range *(2,12)*. Likewise, the 160-fold variation in the proportion of 18:3ω-3 in the yolk lipids of these species had little or no effect on the representation of either 18:3ω-3 or 22:6ω-3 in brain phospholipid at the time of hatch *(2,12)*. The phospholipid fatty acid profiles of heart and skeletal muscle at hatch are slightly more responsive to interspecies differences in egg fatty acid composition. For instance, a comparison between the chicken (*Gallus gallus domesticus*) and the king penguin (*Aptenodytes patagonicus*) indicated that the heart phospholipids of the chicken at hatch contained a 1.3-fold greater proportion of 20:4ω-6, and 20% less 22:6ω-3, compared with the penguin *(4)*. However, these differences are very small compared with the marked disparity in egg fatty acid profiles between these 2 species where, for example, the ω-3:ω-6 ratio in the yolk is 3.0 for the penguin compared with only 0.1 for the chicken *(13)*. It should be noted that the evidence presented above to support the view that different avian species at hatch express similarities in their tissue phospholipid fatty acid profiles relates to species that produce precocial, semiprecocial, or semialtricial young (i.e., where the embryo has achieved a relatively advanced state of development by the time of hatch). A different picture is presented by species that produce altricial young, where the newly hatched chick is much less advanced and lacks locomotory, thermoregulatory, or self-feeding ability. Altricial chicks at hatch have much lower proportions of 22:6ω-3 in their brain and muscle phospholipids compared

with nonaltricial species *(2)*. However, this is explained by the relatively undifferentiated state of brain and muscle in altricial chicks at hatch rather than being an effect of yolk fatty acid composition. Moreover, the proportion of 22:6ω-3 in brain and muscle of altricial chicks increases throughout the nesting period so that, by the time of fledging, these proportions are similar to those found in non-altricial species at hatch *(2)*.

These findings suggest that the expression of functional maturity by a developing tissue is accompanied by the achievement of a characteristic phospholipid fatty acid profile that shows little interspecies variation and is relatively unaffected by the natural species differences in yolk fatty acid composition. Efficient brain function, for example, may require a defined concentration of 22:6ω-3 in the nerve cell phospholipids. To achieve these optimal tissue compositions against a background of widely different yolk fatty acid compositions, the embryos of different avian species have adopted a range of appropriate regulatory mechanisms. For example, embryos of species whose yolks naturally contain relatively low proportions of 22:6ω-3 express a series of compensatory mechanisms involving the preferential uptake and utilization of this fatty acid *(1,5,6)*. Such preferential uptake of 22:6ω-3 does not, however, occur during embryonic development of the king penguin, a bird whose yolk is exceptionally rich in 22:6ω-3 and other ω-3 HUFA *(12,14)*. On the other hand, the embryo of the king penguin is able to efficiently incorporate 20:4ω-6 into the phospholipids of heart, muscle and kidney despite the extremely high ω-3:ω-6 ratio in the yolk of this species *(14)*.

A key point is that the details of these regulatory mechanisms are species specific and have evolved to enable the embryo to cope with a particular yolk fatty acid profile. Any drastic departure from the "expected" yolk fatty acid pattern, as often occurs when birds are bred in captivity, can result in grossly abnormal phospholipid fatty acid profiles in the embryonic tissues, with potential consequences for functional development and viability (*15*, and unpublished data).

1.3. Determinants of Yolk Fatty Acid Composition

We suggest that the natural fatty acid composition of eggs of a particular species results from the interplay between two main factors. Firstly, there is the effect of the natural diet of the female parent. Although many birds can synthesize saturated fatty acids *de novo* from dietary carbohydrate, and can also convert saturates to monounsaturates by δ9-desaturation, they lack the enzymic ability to synthesize ω-3 and ω-6 PUFAs from these precursors. The PUFAs that are incorporated into egg lipids must, therefore, derive ultimately from PUFAs in the diet of the hen. Interconversions of PUFAs in the liver of the hen add further versatility to the yolk PUFA profile. For example, the 22:6ω-3 of the yolk could derive directly from any 22:6ω-3 present in the hen's diet or, alternatively could originate from dietary 18:3ω-3 after desaturation/elongation. Similarly, the 20:4ω-6 of the yolk could derive from dietary 20:4ω-6, as well as from any 20:4ω-6 synthesized from dietary 18:2ω-6. The large number of avian dietary strategies, each providing a characteristic mix of PUFAs, goes a long way to explaining the great diversity of yolk fatty acid profiles.

The fatty acid composition of the yolk is not, however, simply a passive reflection of the fatty acid composition of the diet. Genetic differences among species comprise the other main determinant of the yolk fatty acid profile. The diet simply determines

the pattern of available fatty acids. However, genetic factors that regulate maternal lipid metabolism determine how much of each available fatty acid is incorporated into the yolk precursor lipoproteins. These genetic differences have most likely evolved to transform the fatty acid compositions of the diets into profiles that are more suited to the needs of the embryo. For example, species that have adapted to diets with extremely high or low ω-3:ω-6 ratios are able to partially compensate for this imbalance by preferentially incorporating the under-represented fatty acids into the yolk precursors while discriminating against the incorporation of those present in excess *(13,16)*. Furthermore, dietary fatty acids that are useless or harmful to the embryo, such as certain long chain monounsaturates, may be barred from incorporation into the egg lipids *(13,16)*. Also, the maternal desaturation/elongation pathway is likely to be most highly expressed in species that consume diets rich in EFA but poor in HUFA.

Avian development is therefore characterized by two main phases of selective utilization of fatty acids. The first of these takes place in the female parent where the fatty acid profile provided by the diet is modified for egg formation. The second phase occurs in the embryo, where the egg fatty acids are used selectively to form the tissue phospholipids. The details of both these phases vary among species and represent adaptations to the exploitation of a very wide range of dietary modes during avian evolution. The outcome is that the extreme variation in the mix of fatty acids provided by the different dietary strategies is tempered during the successive maternal and embryonic stages to produce tissue-specific phospholipid fatty acid compositions at hatch that are largely common to all (nonaltricial) avian species. This dramatic convergence attests particularly to the importance of 22:6ω-3 in brain development and to the role of 20:ω-6 in cardiac function.

1.4. Scope of This Chapter

The purpose of this article is to illustrate three general concepts relating to yolk fatty acid composition. First, we consider how dietary differences among avian species in the wild influence the yolk fatty acid profiles. Second, the role of phylogeny in determining egg fatty acid composition is discussed. Third, the effects of domestication and captive breeding on yolk fatty acid composition in a number of avian species are reported. Fatty acid compositions are expressed as wt%. Thus, the weight of each fatty acid as a percentage of the total weight of all fatty acids in the sample is presented. It is well established that the two main lipid classes of the yolk, triacylglycerol and phospholipid, exhibit markedly different fatty acid profiles *(1,10)*. In particular, the ω-3 and ω-6 HUFAs are, in most species, present mainly in the phospholipid fraction, whereas the C18 EFA are often found in both the triacylglycerol and phospholipid of the yolk. Here, we simply present the fatty acid compositions of the total lipid of the yolk, this being the most relevant parameter from the nutritional point of view. Details of the fatty acid compositions of the individual lipid classes can be found in the original papers quoted in the references. The mean values from a number of replicate eggs of each species are shown. Again, details of sample numbers and standard deviations are given in the original references. All eggs collected in the wild were approved by permits from the relevant licensing authorities of that country or territory.

2. INFLUENCE OF NATURAL DIETS ON THE YOLK FATTY ACID COMPOSITIONS OF AVIAN SPECIES IN THE WILD

2.1. Relationship Between the Fatty Acid Compositions of the Diet and the Yolk

The effects of different dietary fatty acid profiles on the fatty acid composition of the yolk have been studied in great detail using the domestic chicken as an experimental model *(9,17–22)*, and the following general principles have emerged. First, the proportion of saturated fatty acids in yolk lipid is largely resistant to dietary manipulation. For example, supplementation of the diet of the hen with tallow, a rich source of saturated fatty acids, does not significantly increase the representation of saturated fatty acids in the yolk. Second, the proportion of monounsaturated fatty acids in yolk lipid is only slightly responsive to dietary supplementation of the hen with oils that are rich in 18:1ω-9 such as canola oil. Taken together, these results show that birds tend to maintain the degree of saturation and monounsaturation in yolk within rather narrow limits. This is true even if the diet is devoid of both saturates and monounsaturates because the bird can synthesize these fatty acids *de novo* from dietary carbohydrate. Third, the representation of the major PUFA in yolk lipid is highly sensitive to their dietary provision. Thus, dietary supplementation of the hen with sunflower oil, a rich source of 18:2ω-6, greatly increases the proportion of 18:2ω-6 in the yolk lipid. Likewise, supplementation with linseed oil, a rich source of 18:3ω-3, dramatically elevates the yolk content of 18:ω-3. Because of the desaturation/elongation reactions in the liver of the hen, supplementation with 18:2ω-6 or 18:3ω-3 also results in significant, but relatively modest, increases in the respective proportions of 20:4ω-6 and 22:6ω-3 in the yolk. A more effective way of enhancing the level of 22:6ω-3 in yolk is to supplement the hen with fish oil or with other rich sources of this HUFA such as certain algae. Thus, the PUFA profile of the yolk of the chicken is highly flexible and can easily be manipulated by diet. In fact, the effect of dietary fatty acids on yolk composition can be predicted quantitatively using appropriate regression equations *(22)*. These general principles, derived from feeding trials in a domesticated species, appear to be broadly applicable to avian species in the wild. As described below, the natural diets of free-living birds certainly have an impact on the yolk fatty acid "signatures" of the various species.

2.2. Avian Dietary Modes and Fatty Acid Supply

There are approx 9000 extant species of birds consuming a very diverse range of diets *(23)*. Thus, depending on the species, birds may be granivorous (consuming grains and seeds), herbivorous (leaves, shoots, grasses), frugivorous (fruits), nectarivorous (nectar), insectivorous (insects), carnivorous (terrestrial vertebrate prey), piscivorous (fish), and so on. These various dietary modes differ greatly in their total fat content and fatty acid compositions.

The main lipid component of grains and seeds is triacylglycerol in which the predominant fatty acid is usually 18:2ω-6. By contrast, the main lipid constituents of grasses, green shoots and leaves are galactolipids present in the thylakoid membranes of the chloroplasts, and these are a rich source of 18:3ω-3. Thus, granivorous diets will provide 18:2ω-6 as the main PUFA, whereas herbivorous diets will mainly provide 18:3ω-3. These differences in dietary PUFA provision between granivores and herbivores are clearly reflected in the fatty acid profiles of their eggs. The key feature of the eggs of

Table 1
Yolk Fatty Acid Compositions Between Granivorous and Herbivorous Birds (wt%)

	Granivores		*Herbivores*		
	Parrot	*Sparrow*	*Ostrich*	*Goose*	*Pheasant*
Σ saturated	30.0	42.6	30.8	33.7	35.3
Σ monounsaturated	31.2	44.4	37.4	42.7	26.8
18:2ω-6	**34.7**	**5.2**	12.1	4.7	9.1
20:4ω-6	3.0	2.0	1.3	1.6	0.8
18:3ω-3	0.4	1.5	**17.5**	**15.8**	**22.7**
22:5ω-3	0.0	1.8	0.0	0.5	0.0
22:6ω-3	0.7	0.8	0.9	1.0	0.9
Σ EFA	35.1	6.7	29.6	20.5	31.8
Σ HUFA	3.7	4.6	2.2	3.1	1.7
Σ PUFA	38.8	11.3	31.8	23.6	33.5
Ratio ω-6:ω-3	34.3	1.8	0.7	0.4	0.4

Notes: Eggs of the pheasant (*Phasianus colchicus*), Canada goose (*Branta canadensis*), and sparrow (*Passer domesticus*) were collected from nests in southern England. Eggs of the ostrich (*Struthio camelus*) were obtained from nests in Zimbabwe. Eggs of the parrot (green-cheeked amazon, *Amazonia viridigenalis*) were a gift from Edinburgh Zoo. Data from *(2,11,24)* and unpublished results. The characteristic PUFAs of each group is emphasized in bold.

the two granivorous species shown in Table 1 is the predominance of 18:2ω-6 as the major PUFA of the yolk lipid, the much lower representation of 18:3ω-3, and the relatively high ω-6:ω-3 ratio (*2*, unpublished data). By contrast, the egg lipids of the three herbivores contain 18:3ω-3 as the main PUFA, with lower proportions of 18:2ω-6, and with ω-6:ω-3 ratios less than unity *(11,24)*. It should be mentioned that the designation of a particular species as granivore, herbivore etc is not always strictly absolute as many species can vary their diet with a range of food items *(23)*. Of the species shown in Table 1, the goose primarily consumes grass, the ostrich eats leaves, shoots and grasses, and the sparrow eats seeds. However, the pheasant has a more diverse diet, eating green shoots but also including seeds and inverterbrates. Eggs of all these species were collected from natural habitats in the wild and the data, therefore, represent the natural fatty acid "signatures" of these species at these locations. The parrot eggs were, however, obtained from a zoo population where the breeding birds were fed on seeds, nuts, and fruit, and it is possible that the fatty acid composition may differ somewhat from the natural situation. A common feature of eggs of both the granivores and herbivores is the great excess of EFA over HUFA, reflecting the fact that plant tissues provide large proportions of the C18 polyunsaturates but little or no longer chain derivatives. The HUFA that are present in these eggs will most likely originate from desaturation/elongation of dietary EFA in the liver of the female parent.

The diets of carnivorous birds contain relatively high proportions of 20:4ω-6, because many of the edible tissues (e.g., muscle, liver, heart, kidneys) of terrestrial vertebrate prey are a good source of this HUFA. On the other hand, fish lipids are noted for their high content of 22:6ω-3 and other ω-3 HUFA, thereby providing piscivorous birds with a high intake of these ω-3 fatty acids. Again, these contrasting dietary fatty

Table 2
Yolk Fatty Acid Compositions of Carnivorous and Piscivorous Birds (wt%)

	Carnivores		*Piscivores*			*Diet of*
	Buzzard	*Kestrel*	*Skua*	*Emperor penguin*	*King penguin*	*King penguin*
Σ saturated	33.9	34.7	31.4	35.4	35.6	22.3
Σ monounsaturated	41.4	39.1	56.2	49.7	48.9	50.0
18:2ω-6	14.0	13.1	2.0	3.3	1.9	1.8
20:4ω-6	**6.2**	**5.3**	2.6	1.7	1.6	0.7
18:3ω-3	1.6	3.9	0.2	0.5	0.1	0.8
20:5ω-3	0.4	0.7	1.1	1.6	3.0	9.3
22:5ω-3	0.2	0.7	0.6	1.0	1.4	0.9
22:6ω-3	1.8	2.3	**5.9**	**5.5**	**5.9**	11.1
Σ EFA	15.6	17.0	2.2	3.8	2.0	2.6
Σ HUFA	8.6	9.0	10.2	9.8	11.9	22.0
Σ PUFA	24.2	26.0	12.4	13.6	13.9	24.6
Ratio ω-6:ω-3	5.0	2.4	0.6	0.6	0.3	0.1

Notes: Eggs of the emperor penguin (*Aptenodytes forsteri*) and the king penguin (*Aptenodytes patagonicus*) were respectively obtained from Antarctic and sub-Antarctic breeding colonies. Skua (*Catharacta skua*) eggs are from the Shetland Isles, UK. Eggs of the American kestrel (*Falco sparverius*) were collected in Saskatchewan, Canada. Eggs of the buzzard (*Buteo buteo*) were provided by the Falcon Facility, UK. Data from *(16,25–27)*. The characteristic PUFAs of each group is emphasized in bold.

acid compositions are reflected in the yolk PUFA profiles of carnivorous and piscivorous birds. Thus, the proportions of 20:4ω-6 in the yolks of the carnivores depicted in Table 2 are notably high compared with the levels in most other dietary groups *(25,26)*. Also, EFA exceed HUFA in the carnivore yolks but not to the same extent as in the yolks of granivores and herbivores. The ω-6:ω-3 ratios in these carnivore eggs are greater than unity. The striking feature of the yolks of piscivores (Table 2) is the very high proportion of 22:6ω-3 as well as the presence of the other ω-3 HUFA, 20:5ω-3 and 22:5ω-3 *(13,16,27)*. For example, the proportion of 22:6ω-3 in the piscivore yolks is about 10 times greater than is found in the yolks of granivores. Most notably, the ω-6:ω-3 ratios in the piscivore yolks are all less than unity. Another salient feature of the piscivore eggs, in contrast to the eggs of most other dietary groups, is that HUFA greatly exceed EFA. Of the species data depicted in Table 2, the eggs of the emperor penguin, king penguin, skua, and kestrel were all collected in the wild and therefore provide the natural egg fatty acid profiles of these species. The buzzards were, however, maintained in captivity, the parent birds being fed on quail and newly hatched chickens, so it is possible that their egg fatty acid profile may deviate from the natural pattern.

Also shown in Table 2 is the fatty acid composition of the diet of the king penguin, as determined by analysis of stomach contents of parents that had returned to feed their chicks *(13)*. This fatty acid composition is identical to that of the fresh food (myctophid fishes) of these penguins. This provides an illustration of how the fatty acid profile of the yolk is influenced by, yet is not simply a passive reflection of, the fatty acid profile of the diet. Clearly, the very high levels of 22:6ω-3 and 20:5ω-3 in the diet of the king

penguin result in the predominance of these ω-3 HUFA in the yolk. Nevertheless, the proportions of 22:6ω-3 and 20:5ω-3 in the yolk are substantially less than in the diet. On the other hand, the proportion of 20:4ω-6 in the yolk is more than twice the proportion in the diet. This provides an example of maternal lipid metabolism attempting to compensate for an extreme ω-3:ω-6 dietary ratio by selectively favoring or discriminating against the incorporation of particular PUFA into the yolk. Presumably, this facilitates the incorporation of the appropriate amounts of 22:6ω-3 and 20:4ω-6 into the phospholipids of specific embryonic tissues.

The skua, emperor penguin and king penguin all feed on marine fish. It is worth noting that the pelican (*Pelecanus erythrorhyncos*) and the cormorant (*Phalacrocorax auritus*), which feed on freshwater fish during the period of egg formation, lay eggs that are rich in 22:6ω-3 but which also contain very high proportions of 20:4ω-6 *(27)*. Although ω-3 HUFA are the characteristic fatty acids of both marine and freshwater fish, the latter often contain high levels of 20:4ω-6 *(28)*, presumably explaining the difference in yolk fatty acid profiles of birds that obtain their food from marine vs freshwater sources.

Most birds of commercial importance, and those kept as pets or for sport, tend to be either granivores (e.g., chicken, parrot), herbivores (e.g., goose), omnivores (e.g., duck, partridge), or carnivores (e.g., falcon). This may obscure the fact that insectivory is by far the most common avian dietary mode. An investigation of the consumption patterns of 168 avian families revealed that 50 of these are primarily insectivorous, with a further 82 that include insects as part of a mixed diet *(29)*. It is well established that 18:2ω-6 and/or 18:3ω-3 are the predominant PUFA of terrestrial insects *(30)*. However, terrestrial insects are generally regarded as being poor sources of HUFA *(30)*. Nevertheless, detectable amounts of HUFA may occur in certain specialized insect tissues *(31)*. Analysis of the fatty acid compositions of 7 families of insects, plus larvae from 2 of these families, indicated that the proportion of 18:2ω-6 ranged from 11 to 36% of total fatty acids, whereas 18:3ω-3 varied from 0.1 to 22% *(32)*. All these samples contained detectable amounts of 20:4ω-6 (0.1–2.6%), and all except one contained some 20:5ω-3 (0.1–3.2%). However, 22:6ω-3 and 22:5ω-3 were not detected in any of these types of insect *(32)*.

Of the five insectivorous species considered in Table 3, four (the red-winged blackbird, mountain bluebird, great tit, and barn swallow) are taxonomically related, belonging to the same avian order, Passeriformes. This is by far the largest avian order, containing over 60% of all bird species. The yolk fatty acid profiles of these insectivorous Passeriformes share many common features. In all cases, the main PUFA is 18:2ω-6, with lesser proportions of 18:3ω-3. All have ω-6:ω-3 ratios greater than unity, but not excessively so. Most notably, these eggs have a unique pattern of ω-3 HUFA. In particular, there is a significant presence of the ω-3 docosapentaenoic acid (22:5ω-3), which forms 2 to 3% of total fatty acids. In each case, the proportion of 22:5ω-3 exceeds that of 22:6ω-3. Eicosapentaenoic acid (20:5ω-3) is also present at 0.5 to 1.9%. The other insectivorous bird shown in Table 3, the ant-eating northern flicker, belongs to a different taxonomic group, the order Piciformes (woodpeckers), and clearly has a fatty acid "signature" that is distinct from the Passeriformes. In particular, 22:5ω-3 is not detected in the eggs of the flicker. This raises the possibility that the characteristic presence of 22:5ω-3 in the Passeriforme eggs is a consequence of phylogeny rather than diet, a view supported by the substantial presence of 22:5ω-3 in eggs of the seed-eating Passeriforme, the sparrow (Table 1).

Table 3
Yolk Fatty Acid Compositions of Insectivorous Birds (wt%)

	Flicker	*Blackbird*	*Bluebird*	*Tit*	*Swallow*	*Diet of swallow*
Σ saturated	30.7	41.4	37.7	31.8	39.0	26.7
Σ monounsaturated	52.5	39.7	42.7	41.5	40.6	43.1
18:2ω-6	6.5	10.4	10.2	18.2	10.3	15.4
20:4ω-6	2.6	2.5	1.3	2.3	2.0	1.0
18:3ω-3	1.1	1.0	5.2	3.7	2.2	11.1
20:5ω-3	1.1	1.0	0.5	0.5	1.9	1.0
22:5ω-3	0.0	2.2	2.0	2.0	3.0	0.0
22:6ω-3	0.4	1.6	0.4	0.4	0.9	0.0
Σ EFA	7.6	11.4	15.4	21.9	12.5	26.5
Σ HUFA	4.1	7.3	4.2	5.2	7.8	2.0
Σ PUFA	11.7	18.7	19.6	27.1	20.3	28.5
Ratio ω-6:ω-3	3.5	2.2	1.4	3.1	1.5	1.4

Notes: Eggs of the northern flicker (*Colaptes auratus*), red-winged blackbird (*Agelaius phoeniceus*) and the mountain bluebird (*Sialia currucoides*) were collected in Saskatchewan. Eggs of the barn swallow (*Hirundo rustica*) and diet samples of this species were from northern Italy. Eggs of the great tit (*Parus major*) were from northern France. Unpublished data. The characteristic yolk PUFA of insectivorous passerines is emphasized in bold.

It should be noted that some of the species shown in Table 3 supplement their diet of insects with other food items. The red-winged blackbird, which is in fact the most abundant avian species in North America, eats considerable amounts of seeds as well as insects. The mountain bluebird mainly eats insects but also consumes a small amount of fruit. The great tit subsists primarily on insects and insect larvae but also eats some seeds and nuts. The barn swallow, however, lives exclusively on small flying insects. The similarities in the yolk fatty acid profiles of these species, despite such dietary variations, are also consistent with a common genetic influence. The interrelation between phylogeny, diet, and yolk fatty acids is further clarified by analysis of the prey items of the swallow (Table 3). The mean fatty acid compositions of the insects collected from the mouths of 10 individual swallows are shown. Clearly, the fatty acid profile of the yolk differs in many respects from that of the diet. Although 18:2ω-6 and 18:3ω-3 are the main PUFAs of the insect prey, small amounts of 20:4ω-6 and 20:5ω-3 are detectable, each forming about 1% of total fatty acids. Thus the presence, if not necessarily the proportions, of 18:2ω-6, 18:3ω-3, 20:4ω-6, and 20:5ω-3 in the yolk of the swallow can be explained by dietary provision. However, both 22:5ω-3 and 22:6ω-3 are notably absent from the diet of the swallow. The presence of these two ω-3 HUFA in the yolk of this species must, therefore, derive from the desaturation/elongation of dietary 18:3ω-3 and/or 20:5ω-3 by the liver of the female swallow. The distinctive fatty acid profile of the yolk of the swallow (and presumably of the other *Passeriformes*), especially the unique representation of 22:5w3, is not therefore a result of diet but is instead a consequence of genetic features related to maternal fatty acid metabolism.

Many avian species are omnivores, consuming both plants and animals. Moreover, a wide variety of items from these two food categories are often exploited. The mix of

Table 4
Yolk Fatty Acid Composition of Some Omnivorous Birds (wt%)

	Coot	*Moorhen*	*Crow*	*Magpie*
Σ saturated	34.8	34.8	35.4	30.3
Σ monounsaturated	50.0	49.7	44.2	46.8
18:2ω-6	6.4	5.5	10.2	9.2
20:4ω-6	2.0	3.3	4.7	4.1
18:3ω-3	3.4	1.5	1.1	2.4
20:5ω-3	0.4	0.6	0.1	1.2
22:5ω-3	1.7	1.1	1.1	1.5
22:6ω-3	2.5	3.3	2.9	3.6
Σ EFA	9.8	7.1	11.3	11.6
Σ HUFA	6.6	8.3	8.8	10.4
Σ PUFA	16.4	15.4	20.1	22.0
Ratio ω-6:ω-3	1.1	1.4	2.9	1.5

Notes: Eggs of the coot (*Fulica atra*) and moorhen (*Gallinula chloropus*) were collected in southern England. Eggs of the American crow (*Corvus brachyrhynchos*) and the black-billed magpie (*Pica pica*) are from Saskatchewan. Data from *(12)* and unpublished.

fatty acid available for vitellogenesis in these species will, therefore, represent the net outcome of the fatty acid profiles of many different dietary items. Because the proportions of the various items in the diet are unlikely to be fixed, the pattern of fatty acid provision will vary with time and between individuals. Hence, a characteristic species-specific yolk fatty acid profile may be difficult to sustain unless genetic factors impose a major controlling influence. The yolk fatty acid compositions of four species with varied omnivorous diets are shown in Table 4. The notable common feature of these profiles is that all the major PUFAs are well represented (*12*, unpublished data). The main PUFA is 18:2w6, and significant amounts of 18:3ω-3 are also present. Moreover, the yolks contain relatively high levels of both 20:4ω-6 and 22:6ω-3, whereas 20:5ω-3 and 22:5ω-3 are also detected. The proportions of EFA only slightly exceed those of HUFA, and the ω-6:ω-3 ratios are generally close to unity. These results suggest that a diverse omnivorous diet endows the yolk with a varied and balanced mix of PUFAs.

The yolk fatty acid profiles of the coot and moorhen are very similar. This is not surprising, firstly because these two species are closely related as members of the *Rallidae* family of the order *Gruiformes* and, secondly, because they consume similar diets of aquatic plants and aquatic invertebrates. Likewise, the American crow and the black-billed magpie are closely related, both being members of the Corvidae family of the *Passeriforme* order. Again, the fatty acid profiles of these two corvids are very similar to each other but are quite distinct from those of the insectivorous *Passeriformes* shown in Table 3: in particular, the levels of 22:5ω-3 in the corvid yolks are far less than the levels of 22:6ω-3. This may result partly from phylogenetic distance because, in the most recent classification, the *Corvidae* are part of the "core Corvoidea" branch of the *Passeriformes*, whereas the four species in Table 3 are all constituents of a separate branch, the "Passerida" *(33)*. There are also similarities in the diets of the crow and magpie, the former consuming grain, seeds, insects, eggs, carrion, fruit and nuts, and the latter selecting a mixture of insects, carrion, rodents, seeds, fruit, eggs, and nestlings.

Data on yolk fatty acid profiles are currently available for only a tiny proportion of the total number of avian species in the wild. Even so, some general principles illustrating the imprint of maternal diet on yolk PUFA composition have emerged, as described in this section. However, the effects of many avian dietary modes remain to be investigated. There are, for example, no details available on the yolk fatty acids of species that exist primarily on molluscs (molluscivores), crustaceans (crustacivores), zooplankton (planktonivores), fruits (frugivores), nectar (nectarivores), fungus (fungivores), lichens (lichenivores), or sap (exudativores). Moreover, the effects within a species of geographical location, dietary variation and habitat degradation have yet to be investigated.

3. PHYLOGENETIC EFFECTS ON YOLK FATTY ACID COMPOSITION

3.1. Information From Domestic Species

There are many studies that have reported direct comparisons between the fatty acid compositions of the diets and the yolks of the chicken, often using feeds with a wide range of PUFA profiles *(9,17,19,20)*. Although these studies have emphatically demonstrated the profound influence of diet on yolk PUFA, they also show clearly that yolk fatty acid composition is by no means an exact replica of the dietary provision. Evidently, maternal lipid metabolism exerts considerable control over the yolk fatty acid profiles.

Further studies have confirmed that these maternal influences on yolk fatty acid profiles differ greatly among species. For example, chickens, turkeys, ducks and geese, maintained on diets with near-identical fatty acid compositions, produced eggs with markedly different PUFA profiles *(15,34)*. In particular, the level of 22:6ω-3 was far higher in the yolk of the chicken than in the yolks of the other three species. On the other hand, the yolks of the goose and duck were greatly enriched in 20:4ω-6 in comparison with the yolks of the chicken and turkey. Because the diets in these studies were devoid of detectable HUFAs, these results suggest genetic differences among species in relation to the efficiency and substrate specificity of the maternal desaturation/elongation pathway.

3.2. Information From Wild Birds

Direct comparisons of the fatty acid profiles of the diets and yolks of both the king penguin (Table 2) and the swallow (Table 3), as discussed in Section 2.2., confirm the view obtained from the studies on domestic species that diet alone cannot account for all the details of yolk PUFA patterns. Moreover, a powerful influence of phylogeny is demonstrated by the distinctive yolk HUFA profile (relatively high levels of 22:5ω-3) displayed by members of the *Passerida* branch of *Passeriformes*, a feature that is not explained by diet (Table 3).

A further example of the influence of a common phylogeny is shown in Table 5. The yolk fatty acid profiles of four species of ducks belonging to the family *Anatidae* of the order *Anseriformes* are very similar, despite the very different dietary preferences of these species *(34)*. The king eider accumulates lipid stores for egg formation by feeding at sea on marine amphipods and mussels, whereas the diet of the lesser scaup consists of freshwater snails, amphipods, and shrimps. By contrast the green-winged teal consumes a range of freshwater invertebrates such as chironomid flies and snails, whereas the gadwall is partly herbivorous, eating filamentous green algae and the leaves and stalks of emergent vegetation. Nevertheless, these duck eggs have a characteristic

Table 5
Yolk Fatty Acid Compositions of Four Species of Duck (wt%)

	Eider	*Scaup*	*Teal*	*Gadwall*
Σ saturates	34.2	34.1	35.7	33.9
Σ monounsats	47.7	52.1	47.6	42.0
18:2ω-6	5.6	3.9	6.6	11.3
20:4ω-6	3.3	2.6	2.2	3.0
18:3ω-3	3.2	2.9	3.1	4.5
20:5ω-3	1.6	1.3	1.1	1.6
22:5ω-3	1.1	1.0	1.3	1.4
22:6ω-3	3.1	2.5	2.6	2.4
Σ EFA	8.8	6.8	9.7	15.8
Σ HUFA	9.1	7.4	7.2	8.4
Σ PUFA	17.9	14.2	16.9	24.2
Ratio ω-6:ω-3	1.0	0.8	1.1	1.4

Notes: Eggs of the king eider (*Somateria spectabilis*) were collected in Nunavut, Canada. Eggs of the lesser scaup (*Aythya affinis*), the green-winged teal (*Anas crecca*) and the gadwall (*Anas streperi*) were from Saskatchewan. Data from *(34)*.

fatty acid "signature," in which all the main PUFAs are well represented. Quantitatively, 18:2ω-6 is the main PUFA, although substantial proportions of 18:3ω-3 are also present. Relatively high levels of both 20:4ω-6 and 22:6ω-3 are present, as well as significant proportions of 20:5ω-3 and 22:5ω-3. The yolk fatty acid profiles of these Anatidae are generally similar to those of the various omnivorous species depicted in Table 4. The duck yolks present a balanced fatty acid pattern, with generally similar proportions of EFA and HUFA and with ω-6:ω-3 ratios around unity. The compositional similarities among the yolks of these four duck species, despite their very different diets, suggests that the common fatty acid "signature" is imposed by phylogenetically-determined maternal effects.

4. THE EFFECTS OF CAPTIVITY OR DOMESTICATION ON YOLK FATTY ACID PROFILES

The diets provided to birds in captivity are rarely identical to the diets that the species select in the wild, particularly in terms of fatty acid composition. Such dietary differences between the two situations can have a dramatic effect on yolk fatty acid profiles. This is illustrated by the four species described in Table 6 where yolk fatty acid profiles for birds breeding in captivity are compared with those of their counterparts in the wild. Pheasants (*Phasianus colchicus*) in the wild consume a mixed diet of green shoots, seeds and invertebrates. The main PUFA in the yolks of the wild pheasants is 18:3ω-3 derived from the green plant material, although 18:2ω-6 from dietary seeds is also well represented. The captive pheasants were fed a commercial wheat-barley diet, designed for this species, that provided 18:2ω-6 and 18:3ω-3 as the only PUFA, at 40 and 4% respectively of the total fatty acids. This 10-fold dietary excess of 18:2ω-6 over 18:3ω-3 massively distorts the yolk fatty acid profile away from the natural "signature" of this species, so that the yolk of the captive birds displays far less 18:3ω-3, and a greatly

Table 6
Comparison of Yolk Fatty Acid Compositions of Wild and Captive Birds

	Pheasant		*Partridge*		*Duck*		*Kestrel*	
	Wild	*Captive*	*Wild*	*Captive*	*Wild*	*Captive*	*Wild*	*Captive*
Σ saturated	35.3	36.9	37.6	37.8	34.0	32.8	34.7	35.5
Σ monounsaturateds	26.8	44.3*	44.8	48.5*	49.0	56.7*	39.1	38.1
18:2ω-6	**9.1**	**15.7***	11.8	11.2	8.6	7.1	**13.1**	**18.5***
20:4ω-6	0.8	0.7	1.8	2.0	2.7	2.3	5.2	5.9*
22:5ω-6	0.0	0.0	0.0	0.4*	0.0	0.3*	0.0	0.0
18:3ω-3	**22.7**	**1.4***	**1.9**	**0.1***	**2.3**	**0.7***	**3.9**	**0.2***
20:5ω-3	0.0	0.0	0.0	0.0	0.8	0.0*	0.7	0.0*
22:5ω-3	0.0	0.0	0.0	0.0	0.6	0.0*	0.7	0.0*
22:6ω-3	0.9	1.0	**1.9**	**0.4***	**2.1**	**0.2***	**2.3**	**1.5***
Σ EFA	31.8	17.1*	13.7	11.3*	10.9	7.8*	17.0	18.7
Σ HUFA	1.7	1.7	3.7	2.8*	6.2	2.8*	9.0	7.4*
Σ PUFA	33.5	18.8*	17.4	14.1*	17.1	10.6*	26.0	26.1
ω-6:ω-3	0.4	6.8*	3.6	27.2*	1.9	10.7*	2.4	14.4*

Notes: Data from *(11,15,26,34)*. Notable PUFA changes are emphasized in bold.
*Significantly different from wild ($p < 0.05$).

increased ω-6:ω-3 ratio, in comparison with the yolks of pheasants in the wild *(11)*. The effects of captivity diets on the yolks of two other species, not shown in Table 6, are very similar to the situation described for the pheasant. Thus, the proportions of 18:3ω-3 in the yolks of wild geese and ostriches are respectively 25- and 8-fold higher than the values for the same species in captivity *(11,24)*.

The partridge (*Alectoris rufa*) in the wild selects a diet consisting of grasses and legumes plus some insects. Partridges in captivity, maintained on a standard grain-based diet that provides 18:2ω-6 as the predominant PUFAs, lay eggs that are relatively deficient in both 18:3ω-3 and 22:6ω-3, when compared with eggs laid by this species in the wild *(26)*. As a consequence, the yolk w6/w3 ratio is greatly elevated by captive breeding. Mallard ducks (*Anas platyrhynchos*) in the wild eat aquatic invertebrates such as snails and chironomid flies. Domestic mallards, maintained on a standard grain-based diet formulated for ducks, produce eggs with far lower proportions of both 18:3ω-3 and 22:6ω-3 than are found eggs of the wild mallards *(15,34)*. Again, captive breeding of ducks results in a dramatic increase in the yolk ω-6:ω-3 ratio, when compared to the natural composition in eggs of free-living mallards. Notably, the eggs of captive partridges and ducks contain detectable amounts of 22:5ω-6, an indicator of ω-3 deficiency.

These "unnatural" yolk fatty acid profiles that result from feeding standard commercial feeds to captive pheasants, geese, ostriches, partridges and ducks are the consequence of providing a granivorous diet to species that are naturally herbivorous or omnivorous. However, such considerations also apply to carnivorous birds. Kestrels in captivity, fed on newly hatched chickens (from eggs laid by hens fed on standard ω-6-rich poultry feed), lay eggs that are relatively deficient in 18:3ω-3 and 22:6ω-3 compared with eggs of wild kestrels *(26)*. Consequently, the ω-6:ω-3 ratio is far greater in eggs of captive kestrels than in eggs of wild kestrels.

5. CONCLUSIONS

Because of the ready availability of poultry eggs, the lipid and fatty acid compositions of the yolk of the domestic chicken have been studied in great detail *(10)*. Such an overwhelming amount of information from just one species can easily lead to the assumption that the fatty acid composition of eggs of the commercially produced chicken (rich in 18:2ω-6 but almost lacking 18:3ω-3) represents the typical avian profile. Clearly, this is not the case. Yolk fatty acid profiles vary tremendously among free-living avian species, reflecting the interplay between diet and the evolutionary adaptation to diet. The ω-6:ω-3 ratio in the yolk of the chicken is typically about 10 and can be much higher in some other captive species *(1)*. However, eggs of wild birds generally have much lower ω-6:ω-3 ratios, despite the wide interspecies differences in dietary strategies. Herbivorous and piscivorous species usually produce eggs with ω-6:ω-3 ratios less than unity whereas, in many omnivores, the ratio approximates to unity. Even the granivorous sparrow has a yolk ω-6:ω-3 ratio of only 1.8. The eggs of many types of free-living birds (e.g., omnivores, insectivores, Passeriformes, ducks) display a varied and balanced mix of ω-6 and ω-3 EFA and HUFA.

For all species where data is available, captive breeding using formulated diets greatly distorts the yolk fatty acid profiles away from the "natural" pattern. Invariably, this is characterized by much higher yolk ω-6:ω-3 ratios resulting from reduced levels of 18:3ω-3, 22:6ω-3 or both. Considering all the species mentioned in this chapter, the mean ω-6:ω-3 ratio in the wild = 1.52 ± 0.99 (mean ± SD; n = 23 species). The mean value of this "3 ratio is some 10 times greater in captivity than in the wild. Moreover, ω-3 PUFA are not the only health promoting constituents that are better represented in eggs of wild birds. The concentrations of antioxidants such as vitamin E, carotenoids, and selenium are also far higher in eggs of wild birds compared with their domesticated counterparts *(11,27,35)*. In this light, the fortification of table eggs with ω-3 PUFA and antioxidants to enhance human health can simply be seen as a return to the natural situation.

Over the last few decades, the global table egg industry has made major achievements in terms of the number of eggs produced. However, egg composition in relation to human health has received less attention. Sufficient information is now emerging to enable us to learn from nature and restore table eggs to a more natural composition that would provide a rich source of ω-3 PUFA and antioxidants for the human diet.

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11 Simultaneous Enrichment of Eggs With PUFAs and Antioxidants

Prospects and Limitations

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Abstract

The recent data indicate that a designer egg enriched in vitamin E, lutein, DHA, and Se can be not only a good nutritional product but also a good vector for the delivery of four essential nutrients vital for human health. A crucial feature of these designer eggs is the synergistic combination of n-3 fatty acids with major antioxidants, vitamin E, lutein and Se, as an important approach to the improvement of the human diet. These eggs will not be able to replace vegetable and fruits as a major source of natural antioxidants and fish products as a source of DHA but can substantially improve the diet, especially in countries like Scotland, significantly contributing to the recommended daily intake of vitamin E, lutein, DHA, and Se. Commercially, it is possible to produce designer eggs enriched with four nutrients or with three, two or one nutrient(s) depending on the consumer demand. As a result, price for the production of such eggs could substantially vary. Therefore the way of egg to the functional food category started successfully and now it is consumer education which is needed to fulfill the idea of using eggs as functional food.

Key Words: Eggs; vitamin E; carotenoids; selenium; antioxidants; omega-3 fatty acids.

1. INTRODUCTION

Recent achievements in biochemistry and molecular biology, together with epidemiological data have changed our thinking about food. It has become increasingly clear that our diet plays a pivotal role in maintenance of our health and that an unbalanced diet can cause serious health-related problems. It seems likely that ω-3 fatty acids and antioxidants are among the major regulators of many physiological processes and therefore a balance between ω-3/ω-6 PUFAs and antioxidants and prooxidants in the diet, gastrointestinal tract, plasma and tissues is an important determinant of the state of our health.

2. DESIGNER EGGS AS A WAY TO IMPROVE HUMAN DIET

For the last few years designer egg production has made a substantial progress in many countries of the world. In particular, ω-3 enriched eggs can be found on supermarket shelves in Europe, The United States, Australia, Malaysia, and Thailand, among others. However, the ω-3 eggs comprise only the first step in the manipulation of egg composition. In particular, natural antioxidants have attracted substantial attention in

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relation to egg quality *(1)*. When considering possibilities of egg enrichment with antioxidants it is necessary to take into account the following:

- Efficiency of nutrient transfer from the feed to the egg. For example, vitamin E and lutein are effectively transferred to the egg, efficiency of vitamin A transfer is much lower and ascorbic acid is not accumulated in the egg at all *(1)*. It is possible to double folic acid concentration in the egg (achieving about 10% RDA), but it seems that further enrichment is limited *(2)*. There are no data available on the flavonoid transfer to the egg.
- Form of nutrient in the diet. Inorganic selenium (Se) in the form of selenite or selenate is characterized by a relatively low efficiency of transfer to the egg. However, organic Se in the form of Se-enriched yeast (Sel-Plex) is transferred to the egg much more effectively. This makes it viable to enrich eggs with Se *(3)*. Either the oil or dry forms of vitamin E are suitable for inclusion into the diet.
- Availability of commercial sources of effective feed forms of antioxidants. Vitamin E (DSM/ROCHE, BASF, etc), organic Se (Alltech, Inc), lutein (Kemin) are commercially available.
- Possible toxic effects of nutrients for the laying hens. For example, vitamin A in high doses in the chicken diet can be detrimental for their health *(4)*, it is not effectively transferred to the egg yolk *(5)* and the elevations that are achieved *(4)* are still far away from the daily requirement in this vitamin. There is a suggestion that vitamin D enriched eggs could be useful for diet improvement for senior citizens *(6)*. In fact, it is possible to commercially produce eggs containing about 40 to 50% RDA in this vitamin *(7)*. However, as with vitamin A, an excess of vitamin D in the hens' diet could be toxic for the bird *(8,9)*. Very high Se doses in the form of sodium selenite could also be detrimental for chicken health and therefore there are legal limits on amounts of Se which can be included into poultry diets. In EU countries, the maximum amount of Se permitted in poultry diets is 0.5 ppm, whereas in the US the permitted level is 0.3 ppm *(3)*. However it is interesting to note that legal limits of Se supplementation are set not because of its toxicity, but because Se is considered as an environmental pollutant.
- Amount of nutrient delivered with an egg in comparison with RDA. If enriched eggs are going to make a significant contribution to human health then arguably they should be capable of delivering an amount of a nutrient comparable with RDA. Our data *(1,3)* showed that with a single egg it is possible to deliver all the daily requirement of vitamin E (15 mg) and 50% of the RDA for Se (30 μg). This became an important marketing tool: "add one egg to the diet and the requirement will be met."
- Established health-promoting properties of nutrients and their shortage in a modern diet. The justification for inclusion of vitamin E into the egg is clear. It is an important component of the antioxidant defences, in many cases diets are deficient in this antioxidant and consumption of high doses of vitamin E (higher than RDA) is beneficial. The same is true for lutein, a carotenoid that has well-established health-promoting properties *(1)* but is often deficient in the modern diet. Furthermore, the health-promoting properties of Se for maintaining health of general public are increasingly being recognized *(3)*. Thus, in most of developed countries Se deficiency is a common feature, while on the other hand, the cancer-preventive properties of Se, and the importance of maintaining the optimal Se status for promoting health, are emerging from recent research *(3)*.
- Possible interactions with assimilation of other nutrients from the egg. When an egg is enriched simultaneously with vitamin E and/or lutein, the lipids of egg yolk could

help antioxidant assimilation. In fact, the amount of lipids in the egg yolk (about 6 g) and their composition (i.e., saturated, monounsaturated and polyunsaturated fatty acids) could provide an ideal milieu for vitamin E and/or lutein absorption by the human intestine. On the other hand, vitamin E, lutein and Se can prevent ω-3 peroxidation during absorption.

- Stability during egg cooking. Vitamin E, lutein and Se are quite stable during egg boiling or frying.
- Effect on appearance and taste. Vitamin E, carotenoids and Se do not affect the organoleptic characteristics of an egg other than helping to prevent the development of a "fishy taint" in ω-3 eggs. Egg enrichment with lutein could be beneficial (in some countries) in terms of consumer preference of deep coloured egg yolk.
- Health claim regulations. Health claim regulations differ substantially from country to country. For example, in Malaysia there are no restrictions on health claims and such claims as "delays the onset of aging" or "increases fertility" can be found on egg box. However in other countries, such as north America and those in the European Community health claims need to be substantiated.

There are also important points in relation to the choice of products for antioxidant enrichment (Table 1) and eggs seem to be an ideal product for this purpose.

3. DIFFERENTIAL RESPONSIVENESS OF EGG COMPONENTS TO DIETARY MANIPULATION

As mentioned above, the levels of some micronutrients of the yolk can easily be increased by supplementation of the diet of the hen, whereas other components are more resistant to change. The explanation for these differences lies in the specific mechanisms whereby the various yolk components are incorporated into the oocyte during vitellogenesis. The lipids of the yolk derive from the uptake of VLDL-type lipoproteins from the hen's plasma. The lipid-soluble antioxidants, vitamin E and carotenoids, are dissolved in the lipid matrix of the lipoproteins. Because of the vast molecular excess of lipid, these lipoproteins can easily accommodate the increased amounts of vitamin E and carotenoids that are provided by dietary supplementation. Thus, the levels of these antioxidants in the egg increase linearly with their concentrations in the parental diet. There are, however, great variations in the efficiency of incorporation of the different types of carotenoids. Lutein, for example, is incorporated into the egg with high efficiency. On the other hand, β-carotene is poorly incorporated into the egg of the chicken, probably because of its conversion to vitamin A in the tissues of the hen. There are species differences here since eggs of many species of wild birds actually contain large amounts of β-carotene. These concepts have been discussed by Surai *(1)*.

Water-soluble vitamins such as thiamin (vitamin B_1), riboflavin (vitamin B_2) and biotin are transported in the hen's plasma, each as a complex with its specific binding protein. These vitamins, together with their binding proteins, are taken up by the oocyte from the plasma and are delivered into the aqueous fraction of the yolk. As discussed by White *(10)*, the levels of these vitamins in the yolk do not increase linearly with dietary supplementation. After an initial increase, the binding proteins in the plasma become saturated with their respective vitamins and so the concentrations of the vitamins in yolk rapidly reach a plateau. Thus, the capacity for enrichment of yolk with these vitamins is restricted by the availability of their binding proteins.

Table 1
Some Characteristics of Food Choice for Se-Enrichment*

The food should be	*Comments*
A part of traditional meals for the population	It would be counter-productive to attempt a change in culturally-based food habits by introducing a new type of food. Emphasis should be given to the possibilities of changing composition of existing foods such as by selenium enrichment.
Consumed regularly in a moderate amount	Because the objective is to deliver the amount of selenium needed to meet RDA it is necessary to choose food which is consumed regularly in moderate amount. Over-supplementation is unnecessary and undesirable.
Consumed by the majority of the population	This is particularly important given that immune function is more likely to be compromised in groups such as children and the elderly.
Affordable	Affordability of food would play an important role in the consumer choice.
Enriched with other health-promoting nutrients that are in short supply in the same population	Examples of minerals critical to health that are frequently deficient include iron and iodine.Vitamin E and lutein are also in short supply in the human diet. This can give a greater improvement in the diet.
Supplying a meaningful amount of the nutrient (e.g., at least 50% RDA)	This is an important point that distinguishes true functional foods from products that include 'tag-dressing' amounts of nutrients for advertising purposes.

*Adapted from ref. *3*.

Although vitamins A and D are lipid-soluble vitamins, they are not located in the lipoprotein fraction of the yolk. Instead, they are partitioned into the aqueous fraction, complexed to their respective binding proteins. Thus, the ability to enhance the levels of vitamins A and D in yolk is subject to the same limitations as described for the water-soluble vitamins. Some elevations of the yolk content of vitamins A and D are, nevertheless, possible because their plasma binding proteins are present in some excess.

Saturated fatty acids, as a proportion of total yolk fatty acids, are resistant to dietary manipulation. Likewise, only modest changes in the proportion of oleic acid (18:1 ω-9) in yolk lipid are achieved when the diet of the hen is supplemented with this fatty acid. Birds tend to maintain the proportions of saturated and monounsaturated fatty acids in yolk within fairly narrow margins, irrespective of diet composition. These constraints result largely from positional specificities. For example, saturated fatty acids are preferred at the sn-1 positions of both the triacylglycerol and phospholipid of the yolk. On the other hand, the PUFA profile of yolk is very responsive to changes in the pattern of dietary PUFA. Thus, supplementation of the hen with oils that are rich in either 18:2 ω-3, 18:3 ω-3 or DHA readily elevates the proportions of these respective PUFA in the yolk. Inclusion of increasing amounts of DHA-rich fish oil in the hen's diet initially produces a linear increase in the representation of DHA in the yolk lipids. In fact, the relationship between dietary DHA and yolk DHA can be described with precision by linear regression equations *(11)*.

There are, however, limits to the incorporation of DHA into yolk lipids. At high levels of supplementation, linearity of the response is lost and the proportion of DHA in yolk approaches a plateau *(12)*. First, this is because the main lipid fraction of yolk, triacylglycerol, normally contains only a trace of DHA, and this proportion is hardly changed by supplementation. The yolk phospholipid fraction is the main site of DHA incorporation, and so this fraction, accounting for only about one-quarter of the total yolk lipid, has to accommodate essentially all of the increase in yolk DHA content that is induced by supplementation. Second, the DHA of yolk phospholipid is located exclusively at the sn-2 position in the molecule. Thus, DHA has access to only half of the potential incorporation sites in yolk phospholipid. Third, the acyl substrate specificity of the acyltransferases that incorporate fatty acids into the sn-2 position of yolk phospholipid mitigates against the unlimited incorporation of DHA at this site. Supplementation of the hen with fish oil enforces the replacement of arachidonic acid (20:4 ω-6) by DHA at the sn-2 position of yolk phospholipid. However, this replacement is never total, as some 20:4 ω-6 is always retained at this position. Also, DHA has to compete with 18:2 ω-6 for esterification into yolk phospholipid. Obviously, for these reasons, it is never possible to produce a yolk in which DHA accounts for a major proportion of the total fatty acids. Nevertheless, by appropriate supplementation, it is possible to raise the level of DHA in yolk lipid to values approaching 4% of the total yolk fatty acids. This represents a four- to five-fold increase over the control value, and is only slightly lower than the proportion of DHA that is achieved in yolks of piscivorous birds such as penguins.

For the purpose of producing eggs that are enriched in health-promoting nutrients, it is extremely fortuitous that ω-3 PUFA, vitamin E and carotenoids all fall into the class of egg constituents that can readily be elevated by dietary means. It is equally providential that yolk Se, uniquely among trace elements, is highly responsive to dietary supplementation, particularly with an organic source of this antioxidant. Again, this reflects the mechanisms of nutrient uptake into the yolk during vitellogenesis, because Se is incorporated into yolk proteins as seleno-amino acids, whereas other trace elements must compete for binding sites on yolk phosvitin. The possibility of producing eggs enriched with a health-promoting combination of ω-3 PUFA and a range of key antioxidants is, therefore, greatly favored by the particular range of uptake mechanisms that have evolved to provision the egg with a wide range of nutrients.

4. SUPER-EGG DEVELOPMENT AND EVALUATION

Recent scientific evidence reinforces the importance of eggs as a healthy food choice. Our attention has been attracted by eggs as a most convenient delivery system for nutrients of our choice: vitamin E (the most abundant lipid-soluble antioxidant), lutein (one of the most important plant carotenoid pigments), Se (a trace element), and DHA (an important long chain PUFA belonging to the ω-3 family).

Based on the results of analyses of eggs obtained from certain wild and free-range birds *(13–15)*, which were characterized by very high concentrations of ω-3 fatty acids, vitamin E, lutein, and Se, it was decided to produce an egg that had enhanced levels of these components, a so-called "super egg." Our idea was to produce an egg containing vitamin E, lutein, DHA, and Se in amounts comparable with the daily requirements of these nutrients in a palatable and visually acceptable form. The main concept was "healthy eggs from healthy birds" because all these four nutrients are as important for the hen's health as for human health.

Table 2
Major Nutrients in a Super Egg

Nutrient in the egg	*Amount (mg)*	*% Recommended Dietary allowances*	*Similar amount provided by*
Vitamin E	19.3	150	100 g corn oil
			150 g margarine
			300 g peanuts
			1 kg butter
			10 kg meat
Lutein	1.91	RDA not known	50 g celery
			100 g green peas
			200 g asparagus
			200 g green pepper
			200 g yellow pepper
Selenium	0.032	50	100 g wheat bread
			150 g brown bread
			500 g meat
			1 kg vegetables
DHA	209	100	49 g sardine
			165 g Atlantic cod
			170 g haddock
			180 g carp

Adapted from refs. *55,56*.

By manipulating the feed of laying hens it was possible to enhance the levels of Se, vitamin E, lutein, and DHA by 7.7, 26.8, 15.9, and 6.4-fold respectively. A single designer egg contained 50% of the RDA of Se, 100% of the RDA of ω-3 HUFA, and 150% of the RDA of vitamin E. It also supplied 1.91 mg lutein (no reference nutrient intake has yet been established) (Table 2).

We conducted a human trial where 44 healthy adult volunteers (24 men and 20 women: minimum, mean and maximum ages respectively were 26, 41.1 ± 1.5, and 59 yr) were recruited and participated in an ethically-controlled trial. The volunteers did not use vitamin E, carotenoid, Se or fish oil supplements and were not using medically prescribed diets or slimming regimes. Subjects were stratified by age and sex and then randomly allocated to either a designer or a commercial table egg per day in a double-blind trial.

Our results indicate that vitamin E is effectively transferred into the egg *(16)* and its concentration in the egg yolk is a reflection of the dietary supplementation. Very high levels of vitamin E can be reached in the egg yolk *(1)* without compromising the productive characteristics of the hen *(17)*. We have chosen an amount of vitamin E/egg (about 20 mg) which provides about 150% RDA and the results of the human trial indicate that the consumption of designer eggs significantly increased the plasma vitamin E concentration over that of the control group and was effective in all treated subjects *(18)* (Table 3). A similar response in plasma vitamin E was found after a 10-wk consumption of antioxidant-enriched margarine providing 31 mg vitamin E/d *(19)*. Plasma γ-tocopherol and vitamin A concentrations were unaffected by treatment. This fact is

Table 3
Initial and Final Plasma Antioxidant Concentrations of the Control and Experimental*Groups (μmol/L)

	Control		*Experimental*	
Parameter	*Before*	*After*	*Before*	*After*
α-tocopherol	25.98	24.95	25.63	30.47
γ-tocopherol	2.10	2.20	2.02	1.98
Lutein	0.21	0.21	0.24	0.45
Vitamin A	2.31	2.17	2.13	2.15

Adapted from ref. *36*.

especially important because the consumption of high levels of ω-3 fatty acids in the form of fish oil by young women was associated with a decrease in vitamin E in plasma and a significant increase in plasma lipid peroxide *(20)*. A similar decrease in vitamin E concentration in plasma was reported for healthy male subjects consuming a daily fish oil supplement *(21)*. Therefore, combination of ω-3 fatty acids with natural antioxidants is an important strategy in human nutrition.

In our human trial *(18)* lutein concentration in plasma significantly ($p < 0.001$) increased (Table 3) in subjects consuming the designer eggs. The increased concentrations of vitamin E and lutein would significantly increase the antioxidant potential of the plasma and help to protect PUFs from peroxidation. Taking into account that vitamin E in the egg yolk is found in the easily digestible α-tocopherol form *(16)* and the highly positive response to vitamin E and lutein supplementation in this study, it seems likely that the bioavailability of these nutrients from cooked egg yolk is quite high. For example, lutein concentration in the plasma increased two-fold (similar to our case) after consumption of a more than five times greater amount of lutein (11.3 mg) from spinach powder *(21)*, but the experiment was shorter (2 wk) compared with 8 wk of egg consumption in this study. Egg yolk contains about 6 g of lipids including saturated, mono- and polyunsaturated fatty acids *(13)* and as mentioned above may be ideal for providing the necessary amount of lipids for mixed micelle formation in the lumen of the human intestine, an important step in lutein and vitamin E absorption *(16,22)*. It has been suggested that increased intake of vitamin E through selection of foods and daily consumption of between five and eight servings of fruit and vegetables rich in carotenoids may reduce risk for cardiovascular disease and improve immune function in later life *(23)*.

In our experiment, when volunteers had adequate Se levels in the diet, consumption of the designer eggs enriched with Se did not affect Se concentration in plasma *(18)*. Se availability from eggs for human needs further elucidation. Possibly, the effect of different dietary sources of Se on its concentration in plasma depends on the Se status of the human. For example, in the case of low-Se status, the consumption of eggs enriched with Se for 3 mo significantly increased serum and hair Se levels *(24)*. Similarly, in Chinese children from the area endemic for Keshan disease (a cardiomyopathy that is closely associated with very low dietary intakes of selenium), Se content in hair increased due to consumption of Se-enriched eggs for 3 yr *(25)*.

Our results showed that 8 wk of intake of control or designer eggs did not alter either total lipid concentration or the proportions of the lipid classes *(18)*. There was no

significant effect of egg consumption on blood pressure, or on total and HDL-cholesterol in the blood. The consumption of DHA-enriched eggs resulted specifically in significantly higher proportions of DHA in each of the human plasma lipid fractions, with no significant changes in any of the other fatty acid components. In plasma phospholipids, DHA was increased 1.3-fold by the designer egg consumption. Increases in the proportion of DHA in the other fractions of plasma lipids ranged from 1.4-fold in cholesteryl ester to 2.3-fold in the plasma triacylglycerol and 1.6-fold in free fatty acid. These increases in plasma DHA affected all treated subjects with the exception of one subject who had had the highest baseline value. It may be suggested that the enhancement of plasma DHA concentrations by eating designer eggs could result in beneficial effects on various health-related parameters such as cardiovascular function, inflammation and immunocompetence *(26–28).*

Our results indicate that two major antioxidant constituents of the egg, vitamin E and lutein, are stable when designer eggs are boiled *(18).* In this experiment, a combination of high levels of two antioxidants vitamin E and lutein in the designer eggs significantly decreased malondialdehyde production during Fe-stimulated lipid peroxidation, in spite of the high content of the highly unsaturated DHA in the egg. Similarly, egg enrichment by vitamin E and carotenoids decreased cholesterol oxidation in egg lipids exposed to nitrogen oxide *(29)* or during egg powder preparation *(30).* Vitamin E enrichment of the egg yolk protects carotenoids from oxidation as well *(30).* Thus, it seems likely that the combination of two antioxidants, namely vitamin E and lutein, accumulated in egg yolk, may improve the storability of the designer eggs compared with normal table eggs, even in the presence of enhanced levels of DHA.

The major advantages of the combination of DHA and antioxidants in the egg yolk are:

- Vitamin E, lutein and Se protect DHA from oxidation during absorption and metabolism in the hen, preventing any "fishy" taste formation in the egg.
- Egg yolk lipids are necessary for the efficient absorption of vitamin E and lutein in human intestine (the 6 g of lipids in egg yolk is ideal for efficient absorption of vitamin E and lutein in the human intestine).
- Lutein interacts with vitamin E and phospholipids, increasing the yolk's anti-oxidant potential and improving egg storability.
- Se, as an integral part of the antioxidant enzyme glutathione peroxidase, protects intestinal membranes against lipid peroxidation during DHA digestion.

These eggs deliver key elements in the diets of pregnant women, the elderly, and young children. The inclusion of such eggs in different processed foods (e.g., mayonnaise and cakes) will increase their nutritional value. The eggs will be of great importance to people living in polluted areas (e.g., Chernobyl, Ukraine) and in areas with very low temperatures (e.g., Polar expeditions), and extreme conditions (e.g., submarine teams).

5. SE-ENRICHED EGGS AS A MODEL FOR FURTHER DEVELOPMENT OF DESIGNER EGGS

Se is a key component of a number of functional selenoproteins required for normal health. It is provided in the Western European diet mainly from bread and cereals, fish, poultry, and meat *(31).* In most diets in the United States, the main food sources of Se

are cereals, meats, and fish *(32).* Selenomethionine represents the major natural form of selenium in feed and food ingredients. Se enters the food chain through incorporation into vegetable proteins as the amino acids selenomethionine and selenocysteine. The British government's defined reference nutrient intake is 75 µg/d for men and 60 µg/d for women *(33).* USA RDI for Se are similar, at 55 µg/d for men and women. An intake of 40 µg/d was suggested as the minimum Se amount required for humans *(34).* A great body of evidence indicates that European intakes of Se are falling. For example, in 1978 Se intake in Britain was 60 µg/d, 7 yr later it was only 43 µg/d, and in 1990 fell to 30 µg/d *(3).* Even in 1997, the average reported Se intake was only 43 µg/d *(35).* Dietary intakes of Se in other countries vary considerably but in many of them intake is still lower than the RDI (Table 4). Reilly *(31)* described more than 40 human diseases and conditions associated with Se deficiency.

Because the Se content in plant-based food depends on its availability from the soil, the level of this element in human foods and animal feeds varies among regions. In general, eggs and meat are considered to be good sources of Se in the human diet. When considering ways to improve human Se intake, there are several potential options. These include:

- Direct supplementation.
- Soil fertilization.
- Supplementation of food staples such as flour.
- Production of Se-enriched functional foods.

It seems likely that the fourth strategy, to produce "functional foods" enriched with Se, deserves more attention *(1,3,6).* Indeed the production of Se-fortified eggs is extremely simple: when Se supplementation of the diet is at a level of 0.4 mg/kg diet in the form of Se-enriched yeast (Sel-Plex), an egg would contain approximately 30 µg of Se which is about 50% of the daily requirement. Se-enriched eggs could easily solve a problem of Se deficiency in countries such as Scotland. This valuable option awaits response from the food industry. However a lack of general knowledge in the field of natural antioxidants as well as in functional food potential benefits is the major limiting factor for wide use of such eggs by consumers.

It seems that Se in eggs is highly available for absorption. For example, a recent clinical trial conducted in the Ukraine showed that consumption of two Se-enriched eggs/d for 8 wk significantly increased the Se level of the plasma of volunteers *(37).* In fact 60 volunteers (30 in control and 30 in experimental group) successfully finished the trial. Eggs consumed in the control group contained 7–9 µg Se/egg and experimental eggs were enriched with Se (28–32 µg Se/egg, levels similar to those found in Columbus eggs). Blood was collected before the beginning and at the end of experimental period and Se was determined in plasma by hydride generation atomic absorption spectrometry with fluorometric detection. The level of Se in plasma of volunteers living in the Kiev area of Ukraine (0.055–0.081 mg/mL) was on the low side of the physiological range and was somehow lower than we reported earlier in volunteers in Scotland *(18).* Consumption of control eggs for 8 wk only slightly increased Se in plasma (0.075–0.085 mg/mL). In contrast, consumption of two Se-enriched eggs daily, which together delivered the daily requirement of between 55 and 65 µg Se, for 8 wk was associated with a significant increase in Se concentration in plasma. Plasma Se reached

Table 4
Low Daily Selenium Intakes in Selected Countries (µg/d)

Country	*µg/d*	*Year reported*
China, Keshan disease area	2–36	1985
China, Keshan disease area	7–11	2001
New Zealand, low-Se area	11	1984
Saudi Arabia	15	1997
Poland	11–40	2000, 2003
UK	12–43	1192, 1195, 1197, 1198, 2003
New Guinea	20	1992
Czech republic	15–50	2003
Nepal	23	1988
Finland before selenium fertilization	26	1987, 1984, 1985
India,vegan low income	27	1997
Egypt	29	1972, 1996
Serbia	30	2001
Slovenia	30	1998
China	26.0–37.2	2000
Croatia	27.3–33.9	1998, 2000
Slovakia	27–38.2	1996, 1998
Belgium	28.4–61.1	1989, 1994
Brazil	28.4–37.0	2004
New Zealand	29–38	1999, 2001, 2004
Sweden	29–44	1991, 2000, 2003
France	29–48	1994, 1994
Turkey	30–36.5	1996, 1997, 2004
UK, 1994	32	1997
UK, 1995	33	1997
England	35	2000
Spain	35	1996
Germany	35–48	1989, 2000
Portugal	37	1990
Denmark	38–47	2000
Italy	43	1985
UK, 1985	43	1997
India, conventional diet	48	1997
Austria	48	2001
Ireland	50	2002
UK, 1974	60	1997

*Se requirement is 55 µg/d (US) and 60 for women and 75 µg/d for men (UK).
Adapted from ref. *3*.

levels of between 0.09 and 0.14 µg/mL, indicating a much improved Se status of the volunteers *(37)*. This is the first clinical trial to prove that Se-enriched eggs could be used as an important vector to improve Se status in countries with low-Se consumption like Scotland or Ukraine.

Table 5
Vitamin E and Carotenoids in Egg Yolk of Birds (µg/g)

Species	*Vitamin E*	*Species*	*Carotenoids*
Wild birds			
Pelican	299.2	Pelican	294.0
Cormorant	150.9	Cormorant	115.7
Lark	156.6	Lark	169.2
Bluebird	206.0	Bluebird	122.3
Blackbird	185.1	Blackbird	185.3
Cowbird	168.7	Coot	131.0
Gannet	216.8	Northern Flicker	107.9
Grebe	228.0	American Kestrel	111.2
Mallard	94.3	Mallard	61.3
Free-range birds			
Free range guinea fowl	31.1	Free range guinea fowl	79.2
Free living pheasant	77.1	Free living pheasant	72.6
Free range chicken	66.3	Free range chicken	75.2
Commercial birds			
Chicken, 10 ppm	33.2	Commercial chicken, wheat-based diet	15.1
Chicken, 100 ppm	205.6	Commercial chicken, maize-based diet	30.2
Chicken, designer diet	1090.5	Chicken, designer diet	106.2

6. ANTIOXIDANTS IN EGGS FROM WILD BIRDS

It seems likely that low Se, vitamin and carotenoid levels in commercially produced table eggs is a reflection of the poultry diets used. As can be seen from data presented in Table 5, carotenoid and vitamin E concentrations in egg yolk from variety of wild species are quite high and in many cases exceed those in commercial table eggs. Furthermore, decreased Se levels in feeds and foods in many cases reflect consequences of our agricultural practices. Therefore, eggs or meat produced by free-range poultry/animals fed on natural feed sources grown on well-balanced soils 100 to 200 yr ago would contain much higher Se concentration than we currently have in many European and Asian countries. Again, by supplementing animal diets with natural organic sources of Se, we are returning back to nature. Our recent data on the Se profile of eggs from various avian species in wild *(15)* confirmed this idea: Se concentration in eggs of 14 avian species in the wild was found to be much higher than that in eggs that derive from commercial poultry production. Our results imply that the Se requirement for birds breeding in captivity will vary among species. Appropriate guidelines could be developed by considering the yolk Se concentration displayed by free-living counterparts of a species. The Se level in the chicken eggs even after organic Se supplementation *(36)* only raised the yolk Se level into the lower end of the range achieved by avian species in the wild, suggesting there may be scope for much higher levels of supplementation for poultry. It seems likely that the Se level which is considered to be the norm

for table eggs is too low to be physiological and this should be studied more in detail in the future. Similar evidence of high-Se concentrations in wild water birds was related to eggs of little egrets, black-crowned night herons, and bridled terns from coastal areas of Hong Kong *(38)*. In tissues of the seabirds from the Barents Sea *(39)*, from Alaska and arctic Russia *(40)*, as well as in bald eagles from Adak Island Alaska *(41)* selenium levels were also several-fold higher in comparison to domestic chickens. Furthermore, high-Se concentrations were reported in eggs from the tree swallow bank swallow and house wren *(42)*. Therefore, Se-enrichment of eggs, meat, and milk is simply the production of naturally designed food ingredients. Indeed, production and commercialization of such organic Se sources as selenized yeast (e.g., Sel-Plex^{TM}) opened a new era in Se supplementation of animals and gave a real chance for producers to meet growing requirements of consumers. What is more, production of these kind of animal-derived foodstuffs is arguably a more consumer-acceptable way to health promotion.

Indeed, it is possible to provide consumers with a range of animal-derived products with nutritionally modified composition in such a way that they can deliver substantial amount of health-promoting nutrients such as Se to improve general diet and help to maintain good health. Therefore, without changing the dietary habits and traditions of various populations it is possible to solve problems related to deficiency of various nutrients, in particular selenium. The consumer will go to the same supermarket to buy the same animal-derived products (e.g., egg, milk and meat), cook and consume them as usual. The only difference will be in the amount of specific nutrients delivered with such products.

7. ANTIOXIDANT-ω-3 ENRICHED EGGS AS FUNCTIONAL FOOD

The concept of healthy food additives arrived from Japan in the 1970s and the term "functional foods" appeared in 1984 *(43)*. At this time, consumers began to view food from a radically different vantage point. This "changing face" of food led to the development of a new area in the food and nutrition sciences *(44)*. The Food and Nutrition Board of the National Academy of Sciences defines a functional food as one that encompasses potentially healthy products providing health benefits beyond that of the traditional nutrients it contains *(45)*. This is in agreement with the data of the 1998 US study from written questionnaires, completed by 2074 respondents indicating that most shoppers believe foods can offer benefits beyond basic nutrition to functional nutrition for disease prevention and health enhancement *(46)*. However, a recent US survey reported that taste is the primary influence on food choice, followed by cost *(47)*. Similarly, in a survey in Ireland, "quality/freshness" of food was the most frequently selected food choice factor (51%) followed by "taste" (43%), and "trying to eat a healthy diet" (36%) *(48)*.

Today, functional foods have received substantial attention *(31,49)* and represent one of the fastest growing segments of the world food industry *(43)*. For example, dairy products and other processed foods, including mayonnaise, margarine and dressings containing DHA *(50)*, as well as ω-3 enriched eggs *(1)*, are already on the market in different countries. Antioxidant-fortified margarine is shown to be effective in the delivery of vitamins E and C as well as α- and β-carotene to humans *(19)*. In the US, annual sales of functional food products comprise around $50 billion *(43)*. In total, functional food has a market share of around 2% in the US food market and is quickly growing *(51)*.

There are three major reasons for the increased interest in functional foods *(45)*: (i) increased health care costs, (ii) recent legislation, and (iii) scientific discoveries.

- Recently, six major targets in relation to functional food science have been identified *(52)*: (i) gastrointestinal functions, (ii) redox and antioxidant systems, (iii) metabolism of the macronutrients, (iv) development in fetal and early life, (v) xenobiotic metabolism and its modulation, and (vi) mood and behaviour or cognition and physical performance.
- In the same review the author has stated that the "health benefit of a functional food will be limited if that food item is not normally part of the diet," Therefore functional foods must remain foods and they must achieve their effects in amounts normally consumed in a diet *(53)*. Eggs have not traditionally been regarded as a functional food, primarily because of concerns about their adverse effects on serum cholesterol levels *(44)*. However recent findings described in this volume indicating that there is little if any connection between dietary cholesterol and blood cholesterol levels, as well as between moderate egg consumption and heart disease, could help to change any bad image of eggs. In this respect, eggs enriched with selenium as well as with a combination of Se, ω-3 fatty acids, vitamin E, and lutein, ideally fit into the category of functional food, enabling substantial improvements in diet quality.

For example, designer eggs could contribute to several aforementioned categories: redox and antioxidant systems (an egg delivers three antioxidant components, including 150% RDA in vitamin E, 50% RDA in Se and a substantial amount of lutein); development in fetal and early life (an egg deliverers a minimal RDA for DHA which is an essential element of the baby brain development), and mood improvement (an egg deliveries 50% of RDA in Se which is considered as having an effect on human mood). Indeed, increased ω-3 PUFA levels in pork and chicken, with simultaneous Se and vitamin E enrichment could have multiple benefits. As in the case of designer eggs, meat enriched with ω-3 PUFA needs increased antioxidant protection. This protection could be provided by increased Se and vitamin E concentrations. In fact there are currently a number of vitamin E-enriched meat products on the market, including sausages and cooked ham *(54)*. It is necessary to underline that Se-enriched eggs, meat and milk could have a positive effect on gastrointestinal function. Lipid peroxidation in the gut is believed to be one of the major contributing factors in the development of various gastrointestinal disorders, which are associated with enterocyte damage, inflammation of the mucosa, and the development of malabsorption.

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12 Egg Composition vs CVD Risk
From Lipid to Endothelial and Inflammatory Hypotheses

Niva Shapira

Abstract

Where the egg debate was initially focused on quantitative-cholesterol aspects, assuming all eggs are the same, recent understanding of atherosclerotic processes has raised questions regarding additional qualitative-composition aspects.

According to the "lipid hypothesis" of atherosclerosis, in which diets high in cholesterol and animal fat were assumed to be high-risk, egg consumption was rigidly restricted. However, accumulating research showing a relatively small effect of dietary cholesterol on blood plasma levels, and the accepted possibility of including eggs in some cholesterol-reducing diets, partially alleviated these restrictions. Recent understanding of contributions by endothelial dysfunction (ED) and inflammation to CVD processes has prompted reconsideration of eggs, with a focus on composition-related risks and/or benefits.

Aside from high cholesterol levels, eggs are rich in unsaturated fatty acids (FA) (≥60% fat), which may reduce blood cholesterol response. Egg composition may further influence blood lipids and endothelial response when high in antioxidants and monounsaturated FA (MUFA), which may reduce the LDL-oxidative response (by ≤30–40% compared to regular high-polyunsaturated FA [PUFA] eggs), or rich in n-3 FA, which may reduce postprandial lipemia (PPL) and resulting ED and inflammation.

"Paleolithic," "wild," and/or traditional "ethnic" eggs, which are higher in MUFA, n-3 FA, and antioxidants than industrially-produced eggs, could offer functional advantages within current egg recommendations, possibly even leading to new guidelines to benefit the general public and/or specific population segments. CVD etiology and risk factors as relate to modern vs. traditional egg composition are discussed in this chapter, and may warrant consideration in future egg recommendations and production.

Key Words: Egg; cardiovascular disease (CVD); cholesterol; n-6 fatty acids (FA); n-3 FA; LDL oxidation; antioxidants; endothelium; postprandial lipemia (PPL); inflammation.

1. INTRODUCTION

Eggs were initially perceived as having the highest nutritional and growth value. But with the increasing incidence of cardiovascular disease (CVD) in modern society, starting from 1950 to 1960, and the emergence of the cholesterol hypothesis, the perception of eggs changed and they were considered a risk food owing to their high cholesterol and "animal fat" content. Whereas initial studies suggested that cholesterol consumption increased the risk, further studies showed its interdependent effect with individual variation (i.e., cholesterol responders vs nonresponders), and/or dietary factors (i.e., high

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saturated vs unsaturated fatty acids, FA). The 40-yr debate regarding cholesterol and eggs was recently reviewed *(1)* and re-evaluation of the effect of dietary cholesterol found the risk to be rather small. As a result, the American Heart Association (AHA) guidelines no longer include a recommendation to limit egg consumption *(2,3)*.

With evolving CVD hypotheses, there is a need to update questions regarding the effects of egg composition (i.e., the potential advantage of high n-3 polyunsaturated FA [PUFA] over high n-6 PUFA eggs), and antioxidant content as related to plasma cholesterol and triglyceride (TG) levels, the inflammatory process, and endothelial function. The scientific backgrounds underlying the CVD hypotheses and paradigms that may be relevant for discussing eggs (i.e., regarding risks and/or benefits that could be affected by their composition) will be discussed here in order to better understand the potential of developing and promoting health-oriented quality eggs.

2. CVD HYPOTHESES

2.1. The Lipid Hypothesis

The lipid hypothesis postulates that an elevation in plasma cholesterol level, mostly in low-density lipoprotein (LDL) particles results in their penetration into the arterial walls, leading to lipid accumulation in smooth muscle cells and in macrophages, the latter creating foam cells. LDL also augments smooth muscle cell hyperplasia and migration into the subintimal and intimal regions in response to growth factors. Here high levels of TG, which interfere with cholesterol removal, may enhance its levels, its damaging effect, and CVD risk *(4)*.

2.2. The Endothelial Injury Hypothesis

This hypothesis *(5)* postulates that cumulative endothelial injury, by various mechanisms that cause loss of endothelial cells, is the earliest stage of the atherogenic process which is followed by aggregation of platelets and adhesion to the subendothelium, monocytes, and T-cells. chemotaxis, platelet and monocyte-derived growth factors that induce smooth muscle cell migration from the media into the intima, their replication, and the synthesis of connective tissue and proteoglycans that form the plaque *(6)*.

2.3. The Endothelial Dysfunction (ED) Hypothesis

ED, which is now thought to be an early physiological event in the atherosclerosis process before morphological changes can be detected, is closely related to lipid risk factors such as hypercholesterolemia (HChol) *(7)* and may predict cardiac events *(8,9)*. Oxidative stress and decreased bioavailability of nitrogen oxide (NO) seem to be common factors that cause ED. Because interventional studies showed recovery from ED to be associated with reduction of lipid-associated risk factors, the new markers of ED, such as reduced endothelium-dependent vasodilation, have become broadly accepted indicators for evaluation of risk assessment, as well as for preventive procedures *(10)*.

2.4. The Inflammatory Hypothesis

Functional properties of the endothelium include active regulation of hemostasis, control of platelet function, coagulation and fibrinolysis, control of vascular tone, endothelial permeability (i.e., to LDL), and medial smooth muscle cell growth *(11)*.

ED occurs under stimuli such as inflammatory cytokines, oxidized LDL (Ox-LDL), and bacterial endotoxins that increase vascular cell adhesion molecules (VCAM). Thus, atherosclerosis was recently attributed to the consequences of chronic inflammation of the large arteries, where ED plays a key role *(12)*.

3. METABOLIC BIOMARKERS VS ENDOTHELIAL FUNCTION (EF)

Several dyslipidemic states were found to promote atherogenesis, in particular the atherogenic lipoprotein phenotype characterized by HTG TG-rich lipoproteins, decreased concentrations of high-density lipoproteins (HDL), increased small dense LDL (SD-LDL), and impaired postprandial lipemia (PPL). These factors have therefore become important in prevention strategies against CVD *(24)*.

3.1. HChol

Total cholesterol and lipoprotein concentrations in children with familial HChol were inversely related to the degree of flow-mediated dilation (FMD), showing that HChol may be associated with the development of ED, before the onset of clinically overt arterial disease *(13–15)*.

3.2. HTG

Brachial artery FMD was significantly diminished in young men with HTG *(16)*. However, acute administration of TG to normal individuals did not impair brachial artery FMD consistently *(16–18)*. Because HTG coexists with insulin resistance, it is likely that it is the latter, rather than the former, which induces ED *(19)*.

3.3. Reduced HDL

Lower plasma concentrations of HDL cholesterol may affect endothelial function independently of LDL cholesterol *(20,21)*. Indeed, infusion of reconstituted HDL cholesterol and restoring the HDL concentration in HTG patients improved EF *(22,23)*.

3.4. Increased LDL

LDL cholesterol has a well-established role in atherogenesis and CVD and recently, improvement in endothelial function (EF) was found upon reducing LDL levels in studies of apheresis and statins *(24)*.

4. OXIDATIVE STRESS VS EF AND INFLAMMATION

Whereas the normal redox state provides normal oxidative metabolism in arterial walls and proper balance between superoxides and NO anions, oxidative stress imposes high superoxide production, NO breakdown, increased expression of adhesion molecules, and leukocyte adhesion to endothelium, which lead to an inflammatory response. Studies to date suggest that a reduction in endothelial NO synthase (eNOS) expression or activity may be detrimental, and that the presence of oxidative stress often dramatically alters the NO function from being beneficial to deleterious *(25)*.

4.1. Oxidized LDL (Ox-LDL)

Steinberg *(26)* put forward the original oxidative modification hypothesis, showing that Ox-LDL is cytotoxic to endothelial cells; moreover, it induces endothelial injury and chemotactically attracts monocytes, promoting their migration, transformation, and retention in the subintimal compartment as macrophages. Thus, Ox-LDL may be responsible for endothelial cell injury, dysfunction, and even their loss in more advanced stages of the lesion. The current oxidative stress hypothesis of atherosclerosis predicts that LDL oxidation is an early, essential event in atherosclerosis and that Ox-LDL contributes to both the initiation and progression of atherosclerosis *(27–29)*.

4.2. SD-LDL

LDL particles are heterogeneous with respect to the sizes, densities, and compositions of lipids *(30)*, from large to SD-LDL. SD-LDL particles are more atherogenic as a result of their higher penetration into the arterial wall, lower binding affinity for the LDL receptor, prolonged plasma half-life, and lower resistance to oxidative stress compared with large LDL particles *(31,32)*. Several studies have reported a two- to three-fold increase in CHD risk among patients with high SD-LDL *(30,33,34)*.

5. NUTRITIONAL RISK FACTORS

5.1. Postprandial Lipemia (PPL) vs Endothelial Dysfunction

PPL was found to be associated with a transient impairment of endothelium-dependent arterial dilatation in adults and in youngsters *(35,36)*. The resulting impairment of FMD was proportional to TG levels, even in young healthy subjects *(37)*. High-fat meals, which cause two times the increase of serum TG after 4 h, and return to baseline after 8 h, induce a transient reduction in blood vessel resistance reactivity after 2 h by 15% *(38)*. The failure of small blood vessels in the heart to increase in size following a high-fat meal as would normally occur after exercise, stress, or a low-fat meal could be detrimental to heart circulation *(37)*. PPL is also associated with a number of adverse metabolic events i.e., the production of atherogenic chylomicron remnants and SD-LDL, reduced HDL levels, and activation of coagulation factor VII *(35)*. Because the PPL phase has an independent role in vascular regulation *(39)*, a fatty meal may become a trigger for coronary syndrome *(21)*, which can be further exaggerated in type 2 diabetes *(40)*.

5.2. PPL vs Oxidative Factors

Reduced EF and blood flow (as indicated by FMD) following a fat load was accompanied by decreased glutathione (GSH) *(41)*, with increased formation of superoxides by neutrophils, whereas vitamin E reversed this reaction. The endothelium-dependent FMD response in diabetics was significantly longer and stronger, which supports the notion that the oxidative burden is involved and correlates with impaired endothelial reactivity during PPL. Recurrent PPL may play an important role in the development and progression of vascular complications, especially in diabetes *(42)*.

5.3. PPL vs Inflammatory Factors

Proxidative-inflammatory mechanisms appear to be involved in PPL-induced endothelial activation, and serum TG was significantly correlated with tumor necrosis factor (TNF)-α, interleukin (IL)-6, and VCAM-1. In addition, a high-fat meal increased the plasma TNF-α, IL-6, intercellular cell adhesion molecule-1 (ICAM-1), and VCAM-1 in normal subjects. In diabetic patients, high-fat and carbohydrate meals significantly increased cytokine and adhesion molecules, which were sustained longer following a high-fat meal, beyond their initially high levels *(43)*.

6. NUTRITIONAL PROTECTIVE FACTORS

6.1. Antioxidants vs EF

Dietary antioxidants improve ED under experimental conditions *(44,45)* of increased ROS *(45)*, such as type 2 diabetes. Oxidized LDL inhibits endothelium-dependent relaxation more potently than native LDL *(46)*, and LDL particles from patients with type 2 diabetes are usually smaller, more dense, and more susceptible to oxidation, and thus more atherogenic than in non-diabetics.

6.2. Antioxidants vs LDL Oxidation

The oxidizability of the lipoprotein particles is highly dependent on dietary and body antioxidants. High amounts of vitamin E (400 mg/d) significantly reduced LDL oxidative susceptibility *(47)* and extremely high amounts reduced LDL oxidation in smokers *(48)*. Moreover, moderate amounts of vitamin E, together with carotenoids, significantly increased the LDL total antioxidant capacity and reduced lag time to oxidation *(49)*. A significant and progressive decrease in tocopherols and carotenoids (i.e., lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and lycopene) was found with increased LDL density, from light to dense LDLs (from LDL1 to LDL5) subspecies, which could underlie the increased oxidizability of SD-LDL *(50)*. LDL oxidation is also dependent on FA composition. Even small changes occurring after every meal might eventually lead to large cumulative effects in those at risk, especially related to highly PUFA-enriched LDL, which is more readily oxidized. Prolonged or exaggerated PPL occurring after successive meals could further lead to the changes in LDL particle density, which are associated with atherogenesis *(51)*.

6.3. N-3 FA vs PPL Response

N-3 FA reduces plasma TG through reduced endogenous very low-density lipoprotein (VLDL) production, enhanced chylomicron clearance, and altered lipoprotein lipase concentration, activity, and affinity for chylomicrons. These facts could perhaps partially explain the reduced PPL response with high n-3 FA *(52)*. The reduced postprandial TG response is emphasized by adding n-3 FA to either high-fat, high-monounsaturated FA (MUFA), or high-saturated FA (SFA) meals *(53)*.

6.4. N-3 FA vs Endothelial Function

Flaxseed oil consumption (20 g/d for 1 mo) has been reported to improve arterial compliance despite increased LDL oxidation. In addition, fish oil (FO) supplements (3 to 10 g/d) consistently ameliorate endothelium-dependent vasodilation (EDV) *(54–56)*. This beneficial

effect of marine n-3 FA might be mediated by increased membrane fluidity of endothelial cells and promote synthesis and/or release of NO *(40,57)*. Interestingly, HChol subjects that were treated with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for 4 months showed a significant improvement in endothelium-dependent flow that mediated the dilation of the brachial artery *(58)*, mostly in people with HTG. The greatest improvement occurred in patients with the greatest increase in red cell membrane EPA and DHA *(37)*.

6.5. N-3 FA vs Inflammation and Hemodynamics

It was already established that increased α-linolenic acid (LNA) from flaxseed oil consumption or FO EPA and DHA can decrease inflammatory cells and soluble adhesion molecules. Recently, moderate amounts of LNA and FO were also found to decrease soluble VCAM-1 (by 16 and 28%, respectively) and soluble E-selectin *(59)*. Moreover, a moderate intake of n-3 FA, in achievable levels, was found to reduce endothelial expression of VCAM-1, E-selectin, ICAM-1, IL-6, and IL-8 in response to IL-1, IL-4, TNF-α, or bacterial endotoxins. The effect paralleled n-3 FA incorporation into cellular phospholipids, and a reduction in VCAM-1 messenger-RNA, indicating a pretranslational effect *(11)*.

6.6. Combined Effect of N-3 FA and Vitamin E

Because n-3 FA is highly oxidizable, vitamin E could indirectly affect the ratios of vasoconstrictive, vasodilator, and aggregatory agents (TXA2 and endothelin/PGI2 and NO, respectively), as well as the expression of adhesion molecules (P-selectin and E-selectin), and thereby influence vascular EF *(60)*. Linoleic acid (LA) and other n-6 FA were found to markedly induce ED and potentiate TNF-mediated endothelial injury, whereas antioxidants protected endothelial cells. This shows the combined interelationships between risk factors, such as high-fat/calorie diets, HTG, and proinflammatory factors (i.e., n-6 FA) vs antioxidants and other protective nutrients that reduce PPL response in atherosclerosis (*61)*.

6.7. Mediterranean Diet vs EF

The Mediterranean diet, which is based mostly on olive oil, pasta, fruits, vegetables, fish, and wine, has an unexpected effect on the FA low rate of CVD. Substitution of n-3 FA-enriched canola oil for the traditional n-9 FA-rich olive oil further reduced CVD events *(62)*. The Mediterranean meals are designed to each contain 900 kcal, 50 g fat from olive, canola, and/or salmon, with or without antioxidant vitamins (C and E) or foods (balsamic vinegar) *(63)*. In spite of the fact that plasma levels of total cholesterol, LDL, apo-lipoprotein B, and P-selectin decreased during both the National Cholesterol Education Program (NCEP)-1 and Mediterranean diets (for 28 d), FMD increased only during the latter *(64)*.

7. EGG COMPOSITION VS CVD RISK

7.1. Egg vs PPL

In the past, increasing plasma cholesterol was the main concern regarding the risk of egg consumption for CVD. Considering the newly recognized dynamic model of events

during PPL, all related dysfunctions were found to be positively correlated with plasma TG, and thus negatively associated with n-3 FA, which are known for having both TG and inflammatory-reducing characteristics. This may suggest that high n-3 FA products, such as n-3 FA-rich eggs may have an advantage over higher n-6 FA eggs, which are the regular type of modern egg.

7.2. Eggs vs EF

In a recent study, Katz et al. (2005) found that EF (as indicated by FMD) remained stable following daily consumption of either 2 eggs or 60 g whole oats over a 6-wk period, despite the fact that total and LDL cholesterol were reduced with the oatmeal regime, but unaffected by the 2 eggs/d regime *(67)*.

7.3. Eggs vs LDL Oxidation

Beyond the effects of eggs on the plasma cholesterol level, intake has been recently shown to increase the oxidation of LDL *(65,66)*. Ox-LDL is assumed to play a key role in the pathophysiology of atherosclerosis *(70)*. Because Ox-LDL is immunogenic, it stimulates the production of autoantibodies to lipoproteins in plasma and in tissues *(71)*. Autoantibodies in atherosclerotic plaques were increased with cholesterol consumption, and correlated with the extent of atherosclerosis in animal models *(72)*. The susceptibility of LDL to oxidation is influenced by the balance between its antioxidative and proxidative tendencies (i.e., PUFA in LDL is readily oxidized, whereas its replacement by MUFA reduces the LDL oxidation). N-6 FA also increases the proinflammatory process, which further facilitates LDL oxidation, whereas oleic acid (MUFA) reduces its oxidation, and its effect on monocyte chemotaxis, adhesion to endothelial cells, and platelet activation *(73,74)*.

7.4. Eggs vs Inflammatory Processes

The addition of 4 eggs/d to the diet was recently shown to increase C-reactive protein (CRP) and serum amyloid A (SAA) in lean insulin-sensitive subjects, and the change in CRP was highly correlated with the change in SAA *(68)*. Both cholesterol and egg feeding was associated with significant increases in HDL cholesterol. The fact that changes in cholesterol and HDL levels were not correlated with CRP or SAA *(68)* may suggest that the inflammatory effect of the eggs may proceed and may not be necessarily correlated with the lipid response. Interestingly, eating eggs increased non-HDL cholesterol *(69)* more in the LIS group than in the obese and/or insulin-resistant group, whose inflammatory marker levels were already elevated at baseline.

8. DESIGNER EGGS: APPLYING THE OLD WISDOM TO MODERN INDUSTRY

Egg modifications may have a significant advantage regarding CVD risks such as PPL and endothelial dysfunction states (i.e., by having improved fatty-acid profiles and antioxidative capacity).

Research studies have shown that it is possible to modify the nutritional values of eggs, especially in the lipid component (Table 1) (i.e., by adding n-3 FA; ALA [18:3] and LCPUFA [EPA, DHA]; vitamins A, D, E; selenium; and iodine) *(75–78)*. It is also possible to increase the content of B vitamins. Eggs may also be enriched with other

Table1
Comparison of the Types of Fats Found in Two Types of Designer Eggs as Well as Generic Shell Eggs *(88)*

	Generic egg	*Reduced fat egg*	*Modified ω-3 egg*
Total Fat (g)	4.5	4.0	4.5
Saturated Fat (g)	1.5	1.2	1.5
Linolenic acid (mg)	17	>50	1000
DHA (mg)	18	>50	100–150
Total omega 3 (mg)	33	>100	100–150
Linoleic Acid (mg)	500	>100	100
Cholesterol (mg)	213	190	213
Vitamin E (IU)	1.1	7.5	6.0

functional components such as β-carotene and lutein *(79)*. In fact, various designer eggs such as the "Columbus eggs," "Eggs Plus," "England's best," and "DHA-enriched eggs" are already commercialized in many countries all over the world *(80–86)* and recently "Bio-ω-3" eggs were featured in Greece *(87)*.

8.1. The Nutritional Benefits of Designer Eggs

Several studies have evaluated the effects of designer eggs on plasma levels of certain nutrients in humans. One egg/d, enriched by 26 times more vitamin E, 16 times more carotenoids, over 7 times the selenium, and 6 times more DHA than generic eggs for 8 wk *(76)* significantly elevated the blood levels of vitamin E, lutein, and DHA, compared with generic shell eggs. In a similar 22-wk study on EPA and DHA-enriched eggs, 7 eggs/wk for the first 20 wk and approx 10 eggs/wk for the last 2 wk significantly elevated the levels of EPA, DHA, and total ω-3 fatty acids in blood. HDL levels gradually rose in the subjects throughout the 24-wk feeding period whereas total blood cholesterol and TG levels were not elevated *(77)*. The author concluded that high EPA and DHA eggs could contribute to the dietary intake of ω-3 PUFA and become an alternative to fish.

8.2. The Functional Benefits of Designer Eggs

The Mediterranean diet, which was recently suggested as the "Gold standard" for heart protection, is characterized by a reduction in oxidative stress (i.e., a reduction in the formation of free radicals, and LDL oxidation) *(89)*. This is attained by high consumption of MUFA and antioxidants from olive oil, fruits, and vegetables *(90)*. Crete-type eggs were found to have higher MUFA and n-3 FA than industrialty-produced eggs *(75,87)*. N-3 FA fortified eggs were recently shown to reduce CRP levels *(69),* and an increased MUFA:PUFA ratio in eggs reduced egg-induced LDL oxidation *(91)*.

8.3 Directions of Designer Eggs

Design of specialized eggs has thus far targeted processes associated with the lipid, inflammatory, and oxidative hypotheses of CVD. Most attain their composition via modification of natural laying hen feed (i.e., substitution of animal fats with grains or common grains with flaxseed) and/or supplementation (i.e., with individual micronutrients, oils [i.e. FO], soluble fibers, and/or fresh vegetal premixes) *(76,86,91–94)*.

8.3.1. High in Antioxidants

The majority of designer eggs yield higher amounts of antioxidants than regular eggs. The most commonly added are vitamin E, carotenoids (β-carotene, lutein), and selenium.

8.3.2. Low in SFA

In these eggs, a significant portion of SFA is usually replaced by PUFA, either n-3 or n-6, elevating the PUFA:SFA ratio. Some may also be reduced in cholesterol.

8.3.3. High in n-3 FA

Many such eggs replace a portion of SFA and/or n-6 FA with n-3 FA (α-LNA and/or DHA), altering both PUFA:SFA (increasing) and n-6:n-3 FA (decreasing) ratios. Some are also fortified with antioxidants.

8.3.4. High in MUFA

Currently, one type of egg is known to have been specifically designed to be high in MUFA, the latter replacing both SFA and PUFA components. It yields a PUFA:MUFA ratio of 0.25:1, and is also fortified with vitamin E, carotenoids, and selenium *(91)*.

9. CONCLUSIONS

The evolution of the CVD paradigm from primarily lipid-dependent to hypotheses incorporating endothelial injury, endothelial function, and inflammation presents continuously changing and even new questions with regard to egg consumption and composition. Eggs are related to all the risk factors, as they contain high amounts of cholesterol, lipids, and pro-oxidative and inflammatory agents. However, the feasibility of modifying egg composition via laying hen feed and the lines of evidence showing the significant impact of egg composition on consumer biomarkers, may suggest that egg modification should first be aimed at reducing the potential for CVD risk, then at improving the consumer's nutritional and health status (i.e., by increasing n-3 FA content for reducing inflammation and TG levels, and/or increasing MUFA and antioxidant content for reducing the LDL oxidation response). Individual variations in metabolic response, as in cholesterol responders vs nonresponders, lean vs obese, and diabetic vs insulin-sensitive subjects with regard to LDL oxidation, endothelial function, and inflammation, may require different recommendations.

Thus, the future of egg consumption will be affected by our understanding of the old wisdom of the wild-type egg, as well as the need to design new eggs according to the new paradigms of CVD risk, as well as to individual variation, to offer the maximum benefit possible for all consumer segments.

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13 Omega-3 Fatty Acids
Studies in Avians

Gita Cherian

Abstract

The research conducted in avians with emphasis on omega (ω)-3 fatty acid enrichment of edible meat portions and the use of fertilized eggs as a unique model for nutrition research is addressed. In a typical Western diet, over 70% of dietary fat is supplied through animal products. Considering the health benefits of ω-3 fatty acids, feeding strategies have been adopted to meet the recommended increased intake. Addition of flax seeds or fish oil is commonly used to manipulate the ω-3 content of poultry foods. The efficacy of flax in increasing the content of long chain ω-3 is limited after 10% inclusion in the bird's diet. Incorporating flax results in an increase in α-linolenic acid (18:3) in triglycerides and long chain (>20-carbon) ω-3 in the phospholipids. Generally, dark meat is rich in α-linolenic acid and white meat is rich in long chain ω-3. However, considering the total fat content of dark meat, which is twice that of white meat, dark meat provides more long chain ω-3 on a portion basis. In oviparous species, the developing embryo is dependent on nutrients stored in the egg for sustaining its growth and development. Thus, hen egg and hatched chick is a unique model to study the role of nutrition in the maternal–fetal system because during development the embryo is in an "isolated" environment not under the influence of nutrients from maternal circulation as in the mammalian system. In addition, the short span of time needed to raise multigenerations of progeny, and considering the similarities that exist between mammalian and avian species in the accretion of long chain polyunsaturated fatty acids during embryonic development makes the avian model a unique tool for nutrition research.

Key Words: Avian model; chicken eggs; meat; omega-3 fatty acids; flax; fish oil.

1. INTRODUCTION

The aim of this chapter is to review the research conducted in avians on ω-3 fatty acids. The health effects of omega (ω)-3 fatty acids and the role of diet is well described in the several other chapters of this book. This chapter will cover the role of poultry foods in providing health-enhancing ω-3 fatty acids to humans and the use of ω-3 fatty acid modified fertilized eggs as a unique model for nutrition research. Research in avians on ω-3 nutrition can be broadly classified as:

1. Those conducted in laying hens as a way of enriching eggs with ω-3 fatty acids and thereby producing eggs with a low ω-6:ω-3 ratio;
2. Those conducted in meat-type chickens and turkeys for increasing the ω-3 content and thereby reducing the ω-6:ω-3 ratio in the edible portion;
3. Those conducted on ω-3 fatty acid modified egg as a unique tool for studying polyunsaturated fatty acid (PUFA) metabolism in the maternal–fetal system; and

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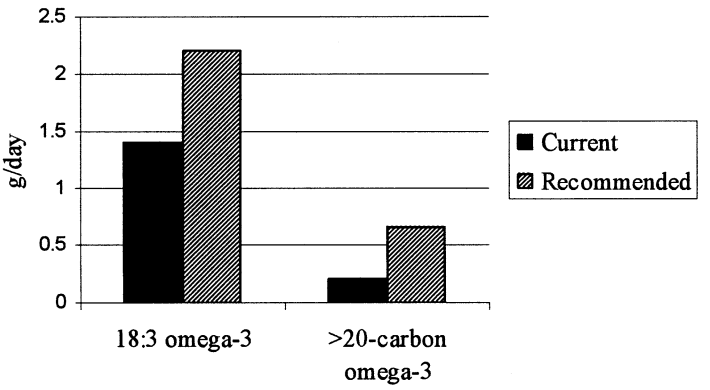

Fig. 1. The current and recommended consumption of ω-3 in the United States diet.

4. Those conducted on metabolic, bird immune health-related aspects and poultry product lipid stability issues.

The major research emphasis on ω-3 in avian nutrition has focused on enriching eggs with ω-3 compared with other aspects. This is evident by the successful marketing of ω-3 fatty acid modified eggs in several countries.

2. CONSUMPTION OF ω-3 IN THE UNITED STATES DIET

The current consumption of ω-3 fatty acids in the United States is 1.4 g α-linolenic acid (LNA, 18:3) and 0.2 g/d of long chain (>20-carbon ω-3) *(1)*. Although no official dietary recommendations have been made in the United States, nutritional scientists suggest including LNA at 2.2 g/d and long chain ω-3 (20:5 + 22:6) at 0.65 g/d *(1)*. Therefore, an additional 0.8 and 0.45 g of LNA and long chain ω-3 is needed in the current United States diet (Fig. 1).

3. ω-3 FATTY ACIDS: WHY POULTRY FOODS?

To accommodate for the 57% (of LNA) and 225% (long chain ω-3) increase in ω-3, alternate dietary sources of ω-3 fatty acids other than marine sources have to be provided. In a typical Western diet, over 53% of dietary fat is supplied through animal products. Therefore, feeding strategies have been adopted to increase the content of ω-3 fatty acids in animal food lipids like meat, eggs, and milk *(2–5)*. However, lipid metabolism in ruminant animals limits the changes in fat composition of milk and ruminant products (e.g., cheese and meat) compared with products from monogastric species (eggs, pork, chicken, and meat). In addition, as consumer preference is to opt for low-fat dairy foods and/or "lean" meat, the availability of ω-3 from ruminant foods will be further limited. Consumer preference, availability, low cost, ease, and versatility in preparation makes skinless poultry meat a major source of animal food protein. Poultry meat accounts for 38% of the consumption of meat and meat products in the United States (calculated from food disappearance data on boneless meat, United States Department of Agriculture (USDA), Economic Research Service, 2004) *(6)* and the

Table 1
Content of ω-3 Fatty Acids in Commonly Used ω-3 Poultry Feed Sources[a]

Oil/oil seed sources	*18:3*	*18:4 n-3*	*20:5*	*22:5*	*22:6*	*Total ω-3*
Oils						
Fish oil	1.3	2.6	14.2	2.7	13.5	34.3
Flax oil	56.0	0.0	0.0	0.0	0.0	56.0
Canola oil	12.0	0.0	0.0	0.0	0.0	12.0
Soybean oil	8.0	0.0	0.0	0.0	0.0	8.0
Marine algae	0.0	0.0	0.0	3.8	7.4	11.2
Oil seeds[b]						
Flax	22.8	0.0	0.0	0.0	0.0	22.8
Canola	5.2	0.0	0.0	0.0	0.0	5.2
Soy	1.6	0.0	0.0	0.0	0.0	1.6
Chia	3.9	0.0	0.0	0.0	0.0	3.9

[a]Reported as weight of total fatty acids (%).
[b]Gram/100 g and is subject to change owing to differences in batch, cultivars, or processing methods used.

average per day consumption of poultry meat is 50.7 g compared with 4.4 g/d fish (weights adjusted for loss) *(6)*. Therefore, poultry meat offers a potential alternate route for increasing dietary ω-3 consumption. In addition, egg consumption is also increasing and is reported to be 253 eggs/person in 2003 compared with 234/person in 1990 *(6)*. Thus, poultry and poultry products are major animal foods consumed in the United States.

4. ENRICHING POULTRY PRODUCTS WITH ω-3s: ROLE OF DIETARY SOURCES

Diet manipulation by incorporating different oils or oil seeds in the chicken diet is the usual way of increasing the ω-3 fatty acid content of eggs and meat. Both land-based and marine-based sources of ω-3 fatty acids are used in poultry diets for ω-3 fatty acid enrichment. The land-based sources provide 18-carbon ω-3 (α-LNA, 18:3), whereas the marine sources provide 20-carbon ω-3 fatty acids such as eicosapentaenoic acid (EPA, 20:5), docosapentaenoic acid (DPA, 22:5), and docosahexaenoic acid (DHA, 22:6). The major land-based sources of ω-3 include flax seeds and flax oil. A lesser amount of ω-3 in eggs could be attained by feeding canola, soy, or chia seeds *(7,8)*. Considering the availability and low price, ground flaxseed is a common LNA source to produce ω-3 fatty acid rich poultry foods.

Fish oil (menhaden, salmon, tuna, and red fish) and marine algae have been used by several investigators as the marine sources of ω-3 fatty acids. Menhaden oil is the most commonly used marine oil for ω-3 fatty acid enrichment. Fish meal in the diet may also contribute to the ω-3 content in eggs. Incorporation of marine sources results in an increase in the content of long chain ω-3 when compared with LNA in flax-fed eggs. Currently, marketed ω-3 specialty eggs are produced by feeding flaxseed, fish oil, and

marine algae. A list of different oils or oil seeds commonly used in poultry feeds and their content of ω-3 fatty acids is shown in Table 1.

5. ω-3 FATTY ACID RESEARCH IN AVIANS

5.1. ω-3 Research in Laying Hens

The effect of diet in modifying egg lipids has been reported as early as 1934 *(9)*. The arrangement of triglycerides and phospholipids formed in the hen liver for yolk synthesis can be affected by dietary modification *(10)*. Therefore, compared with other animal foods, enrichment of egg lipids with ω-3 is fast owing to the high turnover of lipids and lipoprotein in the laying hen *(11)*. Dietary PUFAs can cause major changes in the yolk compared with saturated fats, thus allowing for significant change in yolk ω-3 composition *(11,12)*. Dietary effects of ω-3 are noticed within 9 d of feeding the experimental diet and usually a plateau of incorporation of ω-3 in the egg yolk lipids occurs within 21 d of feeding the ω-3 rich diets *(7)*. Among the different strains of layers used for commercial table egg production, white leghorn hens are more superior in depositing LNA *(13,14)*. Similarly, younger hens less than 35 wk old deposited 25–50% less ω-3 than older birds *(15)*. However, under commercial conditions, companies select specific age range birds (30–50 wk old) to produce specialty designer eggs to minimize any such variation owing to age and to maintain uniformity in the egg ω-3 profile *(3)*.

Addition of ground flax seeds or whole flax seeds (full fat flax seeds) are commonly used for the production of ω-3 eggs. Several authors reported feeding flax in laying hen diets and inclusion of up to 30% of flax has been reported *(3)*. The efficacy of flax in increasing the EPA and DHA content was limited after 10% inclusion *(3)*. In addition, higher levels of flax may also lead to unpalatable flavor in eggs *(16,17)*. Feeding whole seeds may be advantageous in designer egg production as it reduces processing costs and loss of nutrients, and might protect the lipids from oxidative problems resulting from grinding. Nevertheless, grinding did not result in any changes in the deposition of 18:3 in eggs *(18)*. Incorporation of flax oil at 3.5% has been reported to enhance the ω-3 fatty acids to 9.4% resulting in over 500 mg of ω-3 per average egg *(19,20)*. However, owing to the competition of flax oils for human food consumption and availability, using flax oil may not be economically feasible for ω-3 enrichment. A list of reported literature on levels of flax and the total content of ω-3 in chicken eggs is shown in Table 2. It is clear that eggs from hens fed diets containing flax seed could provide over 600 mg of ω-3 PUFA compared with the same amount of ω-3 from a one oz portion of fish *(21)*. Incorporating canola oil or canola seeds could contribute to a lesser extent to the LNA content of eggs. Feeding 16% canola in hen diets resulted in over 235 mg of ω-3 in chicken eggs *(7)* and may be economically feasible owing to cost and availability.

Feeding marine oils may offer the benefit of direct incorporation of long chain EPA, DPA, and DHA into eggs, which are metabolically more important than LNA *(22)*. Menhaden oil is the most commonly used marine oil for ω-3 fatty acid enrichment. Other marine sources include pacific salmon oil, red fish meal, herring meal, and marine algae. Several authors reported varying levels of fish oil (0.5–6%), fish meal (4–12%), and marine algae (2.4–4.8%) in producing ω-3 rich eggs *(3)*. Levels above 3% fish oil and 10% fish meal may lead to flavor problems in eggs because of

Table 2
ω-3 Fatty Acids in Eggs from Hens Fed Flax or Fish Oil[a]

Source and level of ω-3 in hen diet (%)	*LNA (18:3) (mg/egg)[b]*	*Total long chain ω-3 (mg/egg)[b]*	*Total w-3 (mg/egg)[b]*
Flax (%)			
5	114.0	75.2	189.2
8	287.7	99.2	386.9
10	219.3	88.0	307.3
15	270.5	92.2	362.7
16	436.5	94.2	530.7
20	460.0	18.0	478.0
30	604.0	17.0	621.0
Fish oil (%)			
0.5	12.0	96.0	108.0
1.0	8.0	149.0	157.0
2.0	24.0	231.0	255.0
3.0	10.0	168.0	178.0
3.5	5.0	223.0	228.0
4.0	34.0	151.5	185.5
6.0	29.0	164.8	193.8

[a]Adapted from ref. *3,4* and reports the percentage inclusion of flax seed or oil in the hen diet.
[b]Milligram per egg based on an average egg weighing 16 g yolk and 5 g fat.

Table 3
ω-3 Fatty Acids in the White and Dark Meat of Broiler Chickens Fed Diets Containing Flax Seed, Flax Oil, or Fish Oil

Source and level of w-3[a]	*Type of meat*	*Total lipids in meat (g/kg)*	*LNA (18:3) (mg/100g)*	*Long chain ω-3 (mg/100g)*	*Total ω-3 (mg/100g)*
Flax seed (10%)	White	12.0	49.0	120.0	169.0
Flax seed (10%)	Dark	20.2	139.4	175.7	315.1
Flax seed (20%)	White	13.6	95.2	165.9	261.1
Flax seed (20%)	Dark	24.6	253.4	194.3	447.7
Flax oil (5%)	White	11.7	165.0	46.2	211.2
Flax oil (5%)	Dark	24.5	495.0	75.9	570.9
Fish oil (5%)	White	11.2	34.7	560	594.7
Fish oil (5%)	Dark	18.9	71.8	238.9	310.7

[a]Values in parenthesis include the level of ω-3 source in the diet.

a "fishy" taste. However, in countries where fish is eaten more and where fish meal is included in the diets of hens, such 'fish flavor' is acceptable and is more comfortable to consumers *(3)*. Inclusion of marine algae may be important in producing speciality "vegetarian eggs" as well as producing eggs with deeper yellow color owing to the carotenoids present in yolk. Such eggs might find a premium price in some niche

markets. A comparison of ω-3 fatty acids in eggs from hens fed flax, canola, or marine products is shown in Table 2.

5.2. ω-3 Research on Meat-Type Chickens and Turkeys

Meat-type chicken or turkey diets in the United States are predominantly corn, soy, and animal or vegetable fat based, and therefore, the meat from these sources are relatively rich in ω-6 and low in ω-3 fatty acids. In this respect, much work has been done in meat-type chickens to enrich white (breast meat) and dark (leg meat) muscle tissues with ω-3 *(2–4)*. Dietary flax seed, canola seed, menhaden oil, and fish meal were used in several of the reported studies on broiler chickens for ω-3 meat enrichment. However, canola has a limited potential for LNA enrichment compared with flax *(22)*. A comparison of ω-3 in white (breast) and dark (thigh) meat portions from birds fed flax and fish oil is shown in Table 3. As with eggs, menhaden oil increased long chain ω-3 in the breast and thigh meat compared with LNA from flax. Generally, dark meat is rich in LNA and white meat is rich in long chain ω-3. However, considering the total fat content of dark meat when compared with white meat, dark meat provides more of long chain ω-3 on a portion basis. For example, a thigh portion weighing 85 g from broiler chickens fed diets with 15% flax could provide 385 mg of ω-3 compared with 183 mg of ω-3 from half of a chicken breast weighing 180 g from the same chicken. Considering the positive health effects associated with LNA consumption reported in the recent studies (Lyon Heart study, Mediterranean α-LNA enriched Groningen dietary intervention trial [MARGARIN], and those reported by Harper et al., 2006) *(23–25)*, the amount of LNA supplied through poultry products could contribute positively to human health.

Therefore, adoption of technologies to increase ω-3 and thereby reduce the ω-6:ω-3 ratio in chicken meat will make it more attractive to consumers. However, issues regarding lipid oxidation, product stability, added cost of antioxidant supplementation to minimize lipid oxidation, the economics of broiler production such as bird performance (final body weight, feed efficiency), and adverse effects on birds such as diarrhea and reduction in mineral availability reported with feeding flax need to be addressed.

6. HEN-EGG AND HATCHED CHICK (AVIAN MODEL) FOR NUTRITION RESEARCH

6.1. For Metabolic Programming Research

The role of maternal and early diet in fetal physiological programming is getting lots of attention *(26)*. Several of the reported research results on the role of early nutrition and metabolic programming were based on rats. In oviparous species, the embryo is dependent on nutrients stored in the egg for sustaining its growth and development into a healthy hatchling. Thus, the developing chick is in a nutritionally "isolated" and "controlled" environment not influenced by maternal supply of nutrients through the placenta as in mammals. This biologically "self contained" model allows close relationship between nutritive substances and their physiological utilization. The avian model can be a useful tool for studying the "metabolic programming" effect of maternal nutrients on the progeny health. Furthermore, as incubation takes only 21 d, the time-span involved in raising multigeneration progeny with severe deficiency or excess

of a particular nutrient (e.g., essential fatty acids, ω-3, vitamin E, and selenium) is fairly short. In a recent study it was reported that chicks hatched from eggs with high long chain ω-3 diet retained a significantly higher level of long chain ω-3 in the heart, liver, and immune tissue during growth when fed a diet based on LNA *(27–29)*. Similarly, using the avian model, Jiang et al. 1991 *(30)*, reported higher tolerance to dietary cholesterol in chicks hatched from eggs containing high cholesterol. These examples indicate that programming in fetal or embryonic life may result in a lasting response to an environmental stimulus. Thus, nutritional programming has major biological, medical, and animal health significance and the avian model offers a unique tool to explore it in detail.

6.2. For ω-3 Fatty Acid Nutrition Research in Pre- and Term Infants

Considerable similarities exist between mammalian and avian species in the accretion of long chain PUFAs during embryonic development *(31)*. In mammals, the nervous system is the organ with the greatest concentration of lipids after adipose tissue. These lipids are structural and are high in long chain PUFA of the ω-6 and ω-3 series. DHA is the predominant ω-3 fatty acid and arachidonic acid is the major ω-6 fatty acid in the central nervous system of mammals and avians. In the human brain, the last intrauterine trimester is the most active period of brain tissue growth and DHA accumulation *(32)*. During prenatal life, the accretion of long chain PUFA in the human brain is of a quadratic type, the increase being most rapid toward the end of gestation and continuing into early life *(33)*. A similar trend was observed in developing chick embryos preferentially incorporating DHA and arachidonic acid in the brain tissue, during the 3 wk of incubation *(34)*. Thus, despite the obvious developmental difference between mammals and avians, the subsequent usage and metabolism of PUFA is similar, suggesting that the egg and the hatched chick is a unique research model in studying the effect of maternal diet on the metabolism of PUFA in the brain.

7. CONCLUSIONS

The optimal level and form of ω-3 fatty acids in the human diet and in infant formulas has been a focal point of intense scientific scrutiny during the past decade. This has resulted in recommendations to increase the consumption of ω-3 in several countries (e.g., Canada and UK) and to increase the level of ω-3 in infant formula. To achieve the fourfold increase in ω-3s recommended in the United States along with the dietary recommendation to reduce total fat intake, will provide limited options to the consumer. Although fish is considered to be a primary source of ω-3, it may not serve as the primary source of long chain ω-3 owing to availability, cost, and consumer preference. Therefore, manipulating ω-3 in eggs and meat may provide an alternate source to meet the recommended intake. However, eggs are still not a popular food item because of the controversy regarding saturated fat, cholesterol, and its competition with other breakfast food items. Therefore, poultry meat enriched with ω-3 offers potential in enhancing the dietary intake. Other possibilities to increase the dietary consumption include using bakery products with flax flour, mayonnaise, and margarines with ω-3 eggs and LNA-rich oils. Incorporating such foods will increase the dietary intake without altering the dietary habits. Further research is needed to overcome problems in food labeling and

food safety to develop specialty poultry products that will provide nutritious and health-enhancing foods to the consumer.

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14 Chia as a New Source of ω-3 Fatty Acids

Nutritional Comparison with Other Raw Materials and Its Advantages When Producing ω-3 Enriched Eggs

Ricardo Ayerza (h)

Abstract

In pre-Columbian times chia was one of the basic foods of Central American civilizations, following corn and beans in terms of importance, but being more important than Amaranth. Tenochtitlan, the capital of the Aztec Empire, received between 5,000 and 15,000 tons of chia as an annual tribute from conquered nations. Chia seed was not used just as a food, but also offered to the Aztec gods. Today there are ω-3 enriched eggs on the market that are produced by adding flaxseed, chia seed, fish oil/meal or marine algae to the hen's diet. The purpose of this paper is to compare chia with these other raw materials. Available information suggests that the level of ω-3 found in chia eggs could not be reached using flax, fish oil or algae based diets without negatively affecting the hens and/or one or more of the intrinsic characteristics of eggs. In all cases a limiting factor when feeding high percentages of the other ω-3 sources is flavor, smell and/or atypical texture transmitted by these products to the eggs. In the case of flax production also decreases.

Key Words: Chia; ω-3; flax; fish; algae; eggs.

1. A LOST CROP OF THE AZTECS

In pre-Columbian times chia was one of the basic foods of Central America civilizations and followed corn and beans, but was more important than Amaranth. Tenochtitlan, the capital of the Aztec Empire, received between 5,000 and 15,000 tons of chia as an annual tribute from conquered nations *(1)*. Chia seed was not used just as a food, but was also offered to the Aztec gods *(2)*.

The use of chia in pagan religious ceremonies was one reason the Spanish conquistadors tried to eliminate it, and replace it by species brought from the old world *(2,3)*. The conquistadors were close to being successful in their crusade against such New World cultures, not just with chia but with many other crops and customs as well, as they almost disappeared. Corn and beans were an exception, they survived the conquistador's efforts and became two of the most important crops for humanity. Because of its religious use, and maybe because chia was unable to adapt to production under European climatic conditions, it was pushed into a 500 yr period of obscurity *(4)*.

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Table 1
Oil Content and Fatty Acid Composition of Chia and Flaxseed

		*g/100 g**				
Seeds	*Oil %*	*18:3*	*18:2*	*18:1*	*18:0*	*16:0*
Chia	32.8	20.3	6.66	2.36	0.95	2.13
Flaxseed	43.3	25.4	6.32	7.32	1.3	2.25

*Per 100 g of seed.
Adapted from ref. *4.*

Chia survived only in very small cultivated patches in scattered mountain areas of southern Mexico and Central America until a research and development program was initiated in 1991. A group of growers and commercial entities, as well as technical and scientific personnel from Argentina, Columbia, Bolivia, Peru, and the United States began collaborating through the Northwestern Argentina Regional Project. The idea behind the project was not only to provide growers with alternative crops, but also to improve human health by reintroducing chia to human diets as a source of ω-3 fatty acids, antioxidants and fiber *(5)*.

2. ω-3 FATTY ACID SOURCES

At present there are ω-3 enriched eggs on the market obtained by adding flaxseed, chia seed, fish oil/meal, or marine algae to laying hen diets. The purpose of this chapter is to compare the performance of chia to the other raw materials.

Of these raw materials, only flax (*Linum usitatissimum L.*) and chia (*Salvia hispanica L.*) are agricultural crops. These are vegetal species having the highest concentration of ω-3 α-linolenic (ALA = 18:3) fatty acid known (Table 1)*(6–9)*.

The other two sources are of marine origin, that is algae and fish oil/meal. Both contain long chain ω-3 fatty acids, eicosahexaenoic (EPA = 20:5) acid and docosahexaenoic acid (DHA = 22:6). Comparing the four sources shows that the terrestrial ones to have a much higher ω-3 content than the marine sources (Table 2).

3. METABOLISM OF ω-3 FATTY ACIDS IN HUMANS AND POULTRY

The mechanism by which ω-3 fatty acid-rich diets reduce Coronary Heart Disease (CHD) mortality remains controversial. Recent literature discusses the role of different ω-3 fatty acids in human and animals and means of obtaining optimal levels for normal growth and development as well as for the prevention and treatment of CHD and other diseases. ALA cannot be synthesized *de novo*, that is why it is an "essential fatty acid." EPA and DHA can however be formed from ALA through the action of the desaturase and elongase enzymes.

Humans of all ages, including preterms and very likely fetuses, convert ALA to DHA *(10–12)*. This process has been reported for other species as well *(13,14)*. The efficiency of this conversion within species depends on age and diet, whereas between species it is a controversial issue *(15)*. Currently this topic is generating a lot of discussion regarding how to determine the most convenient way to provide ω-3 fatty acids to humans and animals. The main disagreement comes about because of the poor scientific knowledge that describes the biochemistry and physiologic functions of ω-3 fatty acids in general, and of ALA in particular.

Table 2
Fatty Acid Composition of Chia, Flax, Menhaden Fish, and Algae Oils

Fatty acids	*% of total fatty acids*																
oil	*14:0*	*16:0*	*16:1*[1]	*18:0*	*18:1*[2]	*18:2*[3]	*18:3*[4]	*20:3*[4]	*20:4*[4]	*20:5*[4]	*22:5*[4]	*22:6*[4]	*SFA*	*MUFA*	*PUFA*	*ω-6*	*ω-3*
Menhaden[1]	7.9	15	10.5	3.8	14.5	2.2	1.5	0.4	1.2	13.2	4.9	8.6	26.9	25	32	2.2	29.8
Algae[2,*]	17	32	7.8	1.1	4.8	–	–	–	–	0.7	8.4	27.6	50.3	12.6	36.7	–	36.7
Chía[3]	–	6.9	–	2.8	6.7	19	63.8	–	–	–	–	–	9.7	6.5	82.8	19	63.8
Flaxseed[4]	–	5.5	–	1.4	19.5	15	57.5	–	–	–	–	–	6.9	19.5	72.5	15	57.5

[1]ω-7; [2]ω-9; [3]ω-6; [4]ω-3.
*DHA Gold™ *(Schizochytrium sp.)*
Adapted from ref. *4*.

General acceptance has been that ALA fatty acids act as a precursor of the long-chain polyunsaturated fatty acids (PUFAs). This came about because the first epidemiological studies were conducted in populations with high fish intakes, and led to low estimates for ALA requirements *(16)*. Results from recent epidemiological and controlled studies regarding the role of ALA in humans and animal, are changing the way ω-3 sources are thought to work. Evidence is that vegetarians do not suffer from diets lacking DHA *(17,18)* and that ALA supplementation lowered the serum concentration of inflammatory markers in dyslipaedic subjects *(19)*. Additionally medical studies have shown that consumption of ALA is associated with a reduced risk of suffering CHD *(10,18,20–41)*.

The beneficial effects of fish oil have received much attention. However, EPA and DHA fatty acids are easily peroxidized to form hydroperoxides and their secondary degradation products which are thought to be deleterious to cells. There is strong evidence that lipid-derived aldehydes are really cytotoxic and the availability of a cellular GSH-depleting agent is critical for the detoxification of the aldehydes *(42)*. EPA and DHA are more readily oxidized and result in more toxic oxidation products than are linoleic, ALA, and arachidonic acids *(43)*. Scientific evidence shows that EPA and DHA are able to exert beneficial effects in terms of reducing the risk of suffering CHD, only if antioxidative protection against oxidative stress is sufficient to minimize the peroxidative damage of tissue lipids *(44)*.

Oxidation of food lipids is a major concern for both consumers and food manufacturers. If not controlled, oxidation can produce not only food off-flavors (typically known as fishy flavor), but also promote aging and the degenerative diseases associated with aging such as cancer, cardiovascular diseases, cataracts, immune system decline, and brain dysfunction *(45)*.

Chia as an ω-3 source does not require the use of artificial antioxidants such as vitamins. Antioxidant vitamins have been shown to nullify the protector effects of cardiovascular drugs. Research has found that a combination of antioxidant vitamins such as vitamin E, vitamin C, and β-carotene blunt the rise in high-density lipoprotein (HDL) cholesterol levels seen with the drug simvastatin *(46)*. Also, vitamin E has been demonstrated to promote oxidation processes when an upper level is exceeded. The problem is that the low and high limits are too close together, making it very difficult to obtain a correct amount for ingestion when ingredients are mixed for animal feed *(47)*. An advantage of ALA from a plant source over ω-3 fatty acids derived from fish/algae products is that insufficient antioxidant intake does not exist with higher intakes *(15,48)*.

Another concern with recommendations that have been made to increase the intake of EPA as a source of ω-3 fatty acid is the potential for adverse immunological effects resulting from excessive intakes. Moderate to high amounts of EPA, but not ALA, can decrease natural killer (NK) cell activity in healthy subjects *(49)*. NK cells play an important role in host defense against virus infections and in inmunosurveillance against tumor cells *(50)*.

High amounts of DHA can also inhibit the action of Δ_5 and Δ_6 enzymes working on the essential fatty acid, linoleic (ω-6) and ALA (ω-3). Although this action will not affect the total number of ω-3 long chain fatty acids it will cause an imbalance in the ω-6:ω-3 ratio which is considered vital for good function of the human body, including prevention of CHD *(51–55)*.

Table 3
Total ω- Fatty Acid Content and α-Linolenic:DHA Ratios in Eggs Produced by Hens Fed Two Levels of Chia

Fatty acid[1]	*mg/egg*			
diet	*18:3*	*22:6*	*ω-3*	*22:6 : 18:3*
Chia 7%	231	123	354	1:1.9
Chia 14%	438	138	576	1:3.2

[1]Eggs from H&N white hens at 27 weeks age.
Adapted from ref. *14.*

Egg yolks from hens fed chia-enriched diets show a significant increase not only in ALA, but in DHA as well. As in humans, poultry showed the capacity to produce DHA through the desaturation and elongation of ALA, as chia seed lacks DHA (Table 2).

Different organizations involved in human healthcare have made recommendations for the minimum consumption of ω-3 fatty acids which should take place, but these only include ALA the precursor of the other two *(55–59)*. A position paper which The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published in November 2005 gave recommendations for nutrition levels in infant formulae. ESPGHAN set minimum and maximum levels of ALA in infant formulae at 0.3 and 1.2 g/100 kcal, respectively. DHA is listed only as an optional addition, and should not exceed 0.05% of total fat (4.4–6.0 g/100 kcal) intake, arachidonic fatty acid should be at least equal to the concentration of DHA, and EPA should not exceed DHA content *(60)*.

Although there are variations between the recommendations made by nutritionists regarding the relationhip among different ω-3 acids in the diet, especially between DHA and ALA, nutritionists agree that the ALA content must be significantly higher than the DHA content because that is how these fatty acids are found in human milk. Breast milk has a DHA:ALA ratio of, 1:2.1; 1:2.2; 1:2.2; 1:3.3; 1:3.6; 1:3.6; 1:4;1:8; and 1:10.2 in women from Cuba, Germany, France, Nigeria, Argentina, Japan, China, and Nepal, respectively *(61–66)*. Breast milk of women eating a traditional fish-rich diet in East Africa (Tanzania) has a DHA:ALA ratio of 1:1.6 *(67)*. In the United States. DHA:ALA ratios in breast milk from women living in Maryland, Connecticut, and Oklahoma were found to be 1:4.4; 1:2.1 and 1:5, respectively *(68,69)*. Thus in human milk, fatty acid contents vary. In the case of DHA, the observed variations go from 0.04 to 0.25% of total fatty acids *(70)*, and the ALA level was always significantly higher than the DHA content.

Eggs from hens fed 7% chia diets have an ALA:DHA ratio similar to that found in milk of woman from countries where smaller ratios were recorded, that is Cuba, Germany, and France. The DHA:ALA ratios of the eggs (Tables 3 and 4) are almost equal to those (1:1.6) of women consuming a traditional fish-rich diet in East Africa. This ratio has been suggested as being close to that of early *homo sapiens* because of the similarity of their lifelong consumption of East-African fish-based foods *(67)*. The DHA:ALA ratios also are similar to those of eggs from hens raised under free range conditions and consuming green leafy vegetables, fresh and dried fruits, insects and occasional worms *(71)*.

Research has shown that eating regular eggs compared to ω-3 enriched eggs reduces the risk of CHD by lowering plasma triglyceride and cholesterol contents as

Table 4
Cholesterol, Total Fat, Total ω-3 Fatty Acid, α-Linolenic and DHA Content of Eggs Produced by HR White and HR Brown Hens Fed 5 Different Levels of Chia[1]

Chia	*Total fat*	*mg/100 g**			
%	*g/100 g**	*Cholesterol*	*ω-3*	*18:3*	*22:6*
0	9.25	412	95	32	62
7	8.98	318	606	376	231
14	8.62	384	983	812	171
21	9.44	368	1286	1111	175
28	9.29	355	1580	1454	127

[1]Average of eggs from H&N brown and H&N white hens at 27 weeks age.
*Per 100 g of egg, without shell.
Adapted from ref. *14.*

well as blood pressure. Regular eggs, on the other hand, increase these parameters and therefore increase the possibility of CHD *(72–75)*. ALA enriched eggs also significantly reduce platelet aggregation compared with DHA enriched eggs, hence an independent mechanism for dietary ALA-induced reduction of platelet aggregation has been suggested *(76)*.

Reductions in total saturated fatty acids in general, and in palmitic fatty acid (up to 30.6%) in particular, in eggs from hens fed chia-enriched diets compared with eggs from hens fed control diets were significant *(14,77)*. Chia also provided a greater reduction in palmitic fatty acid content than other ω-3 sources when added to the hen diet. Two comparative trials that fed hens ω-3 enriched diets showed eggs from hens fed chia were 16 and 18% lower in palmitic fatty acid than those from hens fed fish oil (Table 5 and 6). Because palmitic and stearic acids are more than 95% of the total saturated fatty acid content of hen eggs, and stearic fatty acid is considered much less hypercholesterolemic than palmitic or not hypercholesterolemic at all *(78–81)*, the reduction in palmitic fatty acid content is more important than a reduction in total saturated fatty acid content in terms of improving the nutritional quality of eggs. Chia has also reduced the palmitic fatty acid content in other animals as well *(13,82)*.

Two trials have shown no differences in palmitic (Table 6) or total saturated (Table 7) fatty acid content of eggs from hens fed either chia or flaxseed enriched diets. As recent research suggests that the reduction of palmitic fatty acid content in egg yolk is feed dependent *(14,83)*, then the lower palmitic content of eggs from hens fed chia and flaxseed enriched diets compared with eggs from hens fed fish oil could be related to the lower palmitic fatty acid content of chia and flaxseed compared with fish oil (Table 2). This beneficial effect gives terrestrial sources of ω-3 fatty acids a dramatic advantage compared with fish and algae products that are very high in palmitic fatty acid, and indicates an additional health advantage for these ω-3-enriched eggs.

4. ω-3 FATTY ACID LEVELS IN COMMERCIAL EGGS

Table 8 lists some of the ω-3 eggs that are available in selected countries of Africa, European Union, and the Americas. The Mega-3 egg, obtained by including 14% chia in the layer diet, contains one of the highest ω-3 contents (1120 mg/100g's of egg

Table 5
Fatty Acid Composition of Egg Yolk Lipids Produced Using Fish Oil (1.6%) and Chia Seed (14%) as Sources of ω-3 Fatty Acid

	Diets			
	Control	*Fish oil*	*Chia*	*Statistical*
Fatty acids	*% of total fatty acid*			*significance*
16:0	28.49[a,1]	28.88[a]	24.17[b]	***
SFA	41.27[a]	41.17[a]	37.01[b]	***
MUFA	32.74[a]	34.83[b]	28.56[c]	***
ω-3	5.05[a]	7.21[b]	16.16[c]	***
ω-6	18.88[a]	14.41[b]	16.32[c]	***
PUFA	23.93[a]	21.61[b]	32.48[c]	***

[1]Means within a row with no common superscript letter are significantly different ($p < 0.05$) according to the Least Significant Difference (LSD).

*** $p < 0.001$.

Adapted from ref. *84.*

Table 6
Cholesterol, Total Fat, Saturated Fatty Acid, and ω-3 Fatty Acid Content of Eggs Produced by Hens Fed 5 Different ω-3 Diets[1]

	Fat	*mg/100 g**							
Diet	*g/100 g**	*Cholesterol*	*16:0*	*SFA*	*ω-3*	*18:3*	*22:6*	*ω-6*	*ω-6:ω-3*
Commercial EB	7.84	368	1719	2500	206	80	122	1062	5.2:1
14% Flax	7.10	354	1580	2180	548	388	138	952	1.8:1
Fish meal	7.92	380	1924	2600	438	160	248	951	2.2:1
14% Chía	7.72	362	1567	2280	948	750	158	1202	1.3:1
Purina layena	8.18	412	1918	2640	204	114	82	1341	6.7:1

[1]Average of eggs from brown and white hens.

*Per 100 g of egg, without shell.

Adapted from ref. *116.*

without shell) of all commercially available eggs (Table 8). However the Mega-3 egg content is far below the potential shown by chia (Table 4), and which has taken place without causing problems in hen development, egg production, or in the flavor, smell, or texture of the eggs *(14,77,83,84)*.

A number of trials were conducted to compare chia and other sources of ω-3 fatty acids. In general they show the advantage of chia over other additives including fish oil and flax for the production of ω-3 eggs (Tables 5 and 6). One recently published research report *(83)* shows the negative effects flax has on egg production when added to a chia enriched diet. The advantage of chia over flaxseed was also shown with broilers as well. This experiment, which compared chia and flaxseed as ω-3 fatty acid sources for poultry meat production, showed significantly ($p < 0.01$) less weight gain in birds fed flaxseed, than in birds fed chia or the control diet *(85)*.

Table 7
Cholesterol, Total Fat, Saturated, ω-6, and ω-3 Fatty Acid Content From Shaver White Laying Hens Fed 4 Different Flax–Chia Diets

Chia	*Flax*	*Fat*	*mg/100 g**						
%	*%*	*g/100 g**	*Cholesterol*	*SFA*	*ω-3*	*18:3*	*22:6*	*ω-6*	*ω-6:ω-3*
0	0	$10.75^{a,b}$	405^{a}	$3630^{a,b}$	199^{c}	81^{c}	$118^{a,b}$	2200^{a}	$11.1{:}1^{a}$
9	5	9.25^{c}	351^{a}	3020^{c}	1009^{b}	846^{b}	164^{a}	2146^{a}	$2.1{:}1^{b}$
11.5	2.5	$9.75^{b,c}$	407^{a}	3080^{c}	1250^{a}	1143^{a}	107^{b}	2276^{a}	$1.8{:}1^{b}$
14	0	9.50^{c}	359^{a}	$3190^{b,c}$	1209^{a}	1112^{a}	97^{b}	2120^{a}	$1.7{:}1^{b}$

*Per 100 g of egg, without shell. Means within a grouping lacking a common subscript differ ($p < 0.05$) according Duncan's Multiple range Test.

Adapted from ref. *83.*

If one compares the ALA content of flax and chia (Table 1) with the amount of ω-3 fatty acid incorporation that takes place in eggs, it can be seen that chia converts much more efficiently, almost 230% that of flax (Table 7). Table 6 also supports this, because chia gave a higher conversion efficiency than flaxseed, producing 217% the deposition/unit of ALA added to the diet, compared with flax. In another trial 119% higher ω-3 fatty acid deposition was found in the meat from broilers fed chia, than in that from broilers fed flaxseed *(85)*. The differences in deposition could be related to the different antioxidants found in flax and chia, and their subsequent influence on fatty acid incorporation. This effect has been suggested by Azcona et al. *(85)*, and by Ayerza and Coates *(83)*. Ajuyah et al. *(86)* observed that addition of antioxidants to broiler diets caused a significant increase in ω-3 fatty acid incorporation into white meat. Interestingly they also observed that the antioxidants did not decrease the negative effects flax had on body growth.

Differences in fatty acid deposition efficiency could also be related to the digestion process of the lipids. Numerous factors are capable of causing variations in intestinal absorption and tissue deposition of fat and fatty acids in nonruminants. These dietary factors include: saturated:unsaturated fatty acid ratio *(87)*; monounsaturated fatty acid plus polyunsaturated: saturated fatty acid ratio *(88)*; and total ω-6:ω-3 fatty acid ratio *(89)*. Digestive utilization of fatty acids varies according to the FA position on the glycerol molecule, hence differences in the ALA positions of chia and flax could explain the higher ω-3 fatty acid incorporation of chia compared with flax *(87,90,91,92)*.

Tables 6 and 7 present incorporation rates of ω-3 fatty acids when whole chia and flaxseed were fed to hens. When ground chia and flaxseed were fed to broilers, ω-3 fatty acid deposition in the meat was only 119% higher per unit of ALA in the broilers fed chia, than that in the birds fed flax *(85)*. Interestingly when whole and ground flaxseed were fed to hens, significant differences in egg yolk flavor were still found *(93,94)*. Given the chia-flax deposition differences obtained between eggs (Tables 6 and 7) and broiler meat it would seem that grinding could reduce the deposition efficiency when used to produce eggs as well.

There was a decrease in deposition differences when flaxseed and chia seeds were ground and fed to poultry. However, given the oxidation and off-flavor problems affecting animal performance and final product quality with flax, combined with the problems of grinding, feeding whole seed is preferred. This gives chia a significant advantage over flax as source of ω-3 fatty acid.

Table 8
Cholesterol, Total Fat and ω-3 Fatty Acid Content of Egg Yolk of Commercially Produced ω-3 Enriched Eggs Obtained From Selected Countries of Africa, European Union, and The Americas

Brand	*Country*	*Cholesterol mg/100 g**	*Total fat g/100 g**	*mg/100 g**					ω-*3 source*
				ω-*3*	*18:3*	*22:6*	*20:5*	*22:6 + 20:5*	
Avine	Brazil	n/a	9	418	n/a	n/a	n/a	n/a	Flaxseed
Columbus	UK	n/a	10.5	1200	n/a	n/a	n/a	n/a	Flaxseed oil
Columbus	France	n/a	10	1500	n/a	n/a	n/a	n/a	Flaxseed oil
Egg plus	USA	430	9	400	200	200	0	n/a	Flaxseed + fish oil
Gold circle	USA	430	9	350	0.00	350	0.00	n/a	Algae
Golden Premium	USA	450	n/a	378	46	239	93	n/a	Fish oil
Eggland's Best	USA	380	8	100	n/a	n/a	n/a	n/a	Canola
Mega-3	Argentina	384	9.2	1120	964	156	0	n/a	Chía
La Piara	Argentina	384	8.9	500	n/a	n/a	n/a	n/a	Chia
Naturegg	Canada	370	9.8	760	n/a	n/a	n/a	n/a	Flaxseed
Maple	Canada	354	9.6	620	n/a	n/a	n/a	n/a	Flaxseed
Born 3	Canada	312	9.8	800	n/a	n/a	n/a	n/a	Flaxseed
Maritime Pride	Canada	354	9.3	620	n/a	n/a	n/a	n/a	n/a
Dietazul	Spain	328	9	266	0.00	n/a	n/a	266	Fish oil
Pitas Pitas	Spain	n/a	n/a	280	0	260	20	n/a	Fish oil
Newstart	South Africa	280	9.5	620	300	250	70	320	Fish oil
SuperEgg	Ireland	n/a	11	228	0	228	0	228	Fish oil
Ovito Piu	Italy	n/a	8.7	350	n/a	280	n/a	n/a	Algae

Notes: n/a: not available.
*per 100 g of egg, without shell.

Ayerza and Coates *(83)* reported that hens fed ALA enriched diets made from a chia-flaxseed mixture produced significantly ($p < 0.05$) different ω-6:ω-3 ratios compared with the control diet. A negative relationship between dietary ALA content and egg ω-6:ω-3 ratio was observed, with the source of ALA having an effect. The higher the chia content, the lower the ω-6:ω-3 ratio. The ratio tended to increase when chia was replaced with flaxseed, however the differences were not statistically significant ($p < 0.05$). This source effect is demonstrated in Table 6, and shows that hens fed chia enriched diets produced eggs having ω-6:ω-3 ratios which were 28 and 41% lower than those of hens fed flaxseed and fish oil, respectively. Neely *(84)* reported ω-6:ω-3 ratios of 3.7:1; 2:1 and 1:1 for egg yolks of hens fed control, fish oil and chia enriched diets, respectively.

Today there is an abundance of evidence supporting the fact that high ω-6:ω-3 ratios of dietary fatty acids, rather than serum cholesterol, are a major risk factor in terms of suffering CHD *(95)*. A reduction in dietary ω-6:ω-3 fatty acid ratios, bringing them closer to the nutritional needs as determined by our genome and (a ratio of 1:1), is considered very important for good health and normal development *(96,97)*.

5. ORGANOLEPTIC CHARACTERISTICS

Eggs produced by hens fed flaxseed have a characteristic (unpleasant) smell, similar to that of hens fed fish oil *(47,98–104)*. In the United States, a study of five cities showed that consumers generally were reluctant to consume eggs smelling/tasting of fish *(105)*. The absence of atypical organoleptic characteristics in eggs produced by hens fed chia represents a significant advantage for this grain, compared with flax and fish by-products *(77,83,106)*.

The difference in organoleptic characteristics of eggs produced using flax and chia could result from the powerful action of the antioxidants in chia which are absent in flax *(107–110)*, and/or to the interaction between other components in flax and bird physiology *(111)*. In the case of fish oil the smell results from the greater instability of DHA and EPA than ALA, combined with an absence of antioxidants capable of preventing degenerative processes *(112,113)*.

In the case of marine algae available literature reports no fish smell or taste in eggs produced using this product as a feed. However, no published scientific papers have been found which support this statement. An indirect reference about off-flavors in eggs produced by hens fed algae is contained in a nonscientific paper written by Abril et al. *(114)*. They mention that including up to 1% algae in laying hen diets did not significantly decrease overall egg acceptability in terms of aroma and/or flavor. Although no scientific information appears to be available to support or refute the presence of off-flavors, the high content and known instability of DHA would logically lead one to assume that algae would transmit undesirable organoleptic characteristics to eggs when large quantities are consumed by hens.

Not all, but some, marine algae have shown antioxidant activity related to total polyphenol content. It has been suggested that polyphenol could prevent oxidative damage to important biological membranes. However, commercially produced algae shows very low antioxidant capacity. The decrease could be related to drying (50°C for 48 h) of the algae during commercial production. Jimenez-Escrig et al. *(115)* reported that

processing (drying) and storing decreases the antioxidant content of fresh algae. Thus the difference in shelf life between chia and algae may be associated with the difference in the quantity and/or quality of natural antioxidants in each.

In summary, several studies provide solid evidence that including more than 5% flaxseed, 1.5% fish oil, or 1% algae in laying hen diets will result in a significant decrease of egg acceptability in terms of aroma and/or flavor. It is possible, however, to include up to 30% chia in the diet without encountering any negative consumer preferences. This means maximum ω-3 fatty acid enriching potentials (mg/egg) of: 175 for algae, 207 for fish oil, 214 for flaxseed, and 986 for chia seed without affecting egg organoleptic characteristics *(14,93,99,106,114)*.

6. CONCLUSION

Available information suggests that none of the levels of ω-3 enrichment that chia can produce could be reached using flaxseed, fish oil or algae based diets without strongly affecting hen performacnce and/or one or more of the intrinsic characteristics of eggs. The limiting factor for utilization of high percentages of available ω-3 sources, with the exception of chia, is change in the flavor, smell and/or texture transmitted by these products to the eggs. Also in the case of flaxseed, egg production would be negatively affected.

Chia, when used as feed for laying hens holds great potential. This is true not only in producing eggs having a high level of ω-3 fatty acids lacking off-flavors and without affecting production parameters, but also by producing eggs having lower saturated fatty acid contents and better ω-6:ω-3 ratios than those produced by hens fed other ω-3 fatty acid sources.

Modern science thus explains why chia was one of the four most important foods not only for the Aztec and Mayan civilizations, but for millions of other Mesoamerica's inhabitants as well. As a result, chia seed has become an alternative to flaxseed and marine sources for the production of more acceptable eggs for the health conscious consumer.

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15 Evaluating the Biological Activity and Effects on Human Health of Fish Oil and Its Omega-3 Fatty Acids

Adrian S. Dobs and Daniel Edelstein

Abstract

This chapter reviews the background, chemistry and specific efficacy of fish oils, specifically ω-3 fatty acids in the treatment and prevention of disease. Epidemiologic evidence appears strong showing its protective effects, although clinical trials with definitive data are still deficient. The mechanism by which ω-3 fatty acids may work on the nervous system and its anti-inflammatory effects are presented. Efforts should be made to ensure dietary intakes should provide sufficient amounts of these essential fatty acids through moderate intake of cold water fatty fish.

Key Words: Fish oil; ω-3 fatty acids; health promotion; chronic disease; cancer; metabolism; diabetes; arthritis; Alzheimer's; depression; essential fatty acids; growth and development; intake recommendations.

1. HISTORICAL BACKGROUND

Fish oil was first recognized for its health benefits in 1937, when medical researchers documented that the Inuit populations of Canada were virtually free of cardiovascular disease (CVD), hyperlipidemia, diabetes, and cancer, whereas at the same time these disorders were common across Europe and North America *(1)*. In as early as 1936, Canadian physician Dr. Rabinowitch reported

> *"If there was a serious health problem amongst the Inuit, I was not aware of it. My interest was primarily in the alleged absence of diabetes, cancer and atherosclerosis and the possible relationship between such absence and the peculiar dietary habits of these people."*

These peculiar dietary habits of the Inuit are that they eat an extremely high fat diet, (approx 39% of their calories from fat), which is derived mostly from fish consumption. This led other researchers to discover that Eskimos in Greenland also had an unusually low rate of coronary heart disease even though they too ate a diet very high in fat *(2–4)*. However, when compared with the Danish people who also consume a similar amount of fat (42% of calories), the Danes experienced nearly ten times the death rate from coronary heart disease (CHD) compared with that of the Inuits *(5,6)*. This stark contrast was attributed to the Inuit's consumption of high levels of protective ω-3 fatty acids, whereas the Danes consumed mostly saturated and trans fats with high levels of

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arachadonic acid. From these initial studies and comparisons, it became quite clear that the quality of the fat (i.e., ω-3 content) may be much more important than quantity in promoting health and preventing disease.

Since these initial reports were made, fish oil and its ω-3 fatty acids have been studied extensively, from preliminary research with animal models and tissue cultures to large controlled clinical trials. Tremendous progress has been achieved in our understanding of the physiological and metabolic effects of ω-3 fatty acids on health promotion and disease prevention and treatment. Specifically, the simple consumption of ω-3 fatty acids from fish may play a critical role in preventing and in some cases treating many of today's most common and distressing health concerns, most notably:hyperlipidemia, atherosclerosis, CHD, arthritis, cancer, cachexia, as well as certain psychological conditions.

2. CHEMISTRY AND DIETARY SOURCES OF ω-3 FATTY ACIDS

α-Linolenic acid (ALA; 18;3n-3), docosahexaenoic acid (DHA; 22:6n-3), and eicosapentaenoic acid; (EPA; 20:5n-3) are the most common dietary ω-3 fatty acids. ω-3 fatty acids are considered essential fatty acids (EFAs), because humans are unable to desaturate the ω-3 bonds, and therefore must be obtained from dietary sources. ALA is found in vegetables nuts and seeds, and is most concentrated in flaxseed, walnuts, purslane, canola seeds, chia seeds, lettuce, soybeans, cauliflower, and spinach *(7)*. EPA and DHA on the other hand, are found almost solely in certain types of fish that feed on microalgae and other single-celled marine organisms which are able to synthesize these fatty acids *(8)*. EPA and DHA move up the food chain via plankton and algae to concentrate in fish and eventually make their way to marine and terrestrial mammals. Oily cold water fish such as salmon, herring, tuna, and sardines are the best sources of EPA and DHA, whereas low-fat fish such as cod, flounder, and haddock are poor sources, as can be seen in Table 1. In addition to the amounts of DHA and EPA, this table also shows the total fat content and the total EPA/DHA content expressed as a percentage of the total fat, which may be useful for those trying to maximize their ω-3 consumption while avoiding excessive dietary fat intake. Notice that even though farmed fish have a similar EPA/DHA profile to that of their wild counterparts, the total fat content of farmed fish tends to be significantly higher and often contains high levels of undesirable saturated fats and contaminants *(9,10)*.

3. ω-3 FATTY ACID METABOLISM AND METABOLIC CONVERSION EFFICIENCY

A topic of much debate is the dietary essentiality of ALA and whether it can affect cardiovascular and neural health as significantly fish oil's EPA and DHA *(11)*. Although ALA from vegetable and terrestrial sources can provide a significant source of dietary short chain ω-3 fatty (EFA) acids, its overall conversion to active forms of the longer chain ω-3 fatty acids (HUFA) EPA and DHA is severely limited and inefficient, and depends greatly on the amount of dietary linoleic acid (LA; 18:2n-6) consumed *(12–14)*. Figure 1 shows the metabolic pathway by which ALA is converted to EPA and DHA, as compared with the metabolic processing of LA. It is important to note that the first step in the metabolic conversion of both LA and ALA require the

Table 1
Approximate Levels of EPA + DHA in 3 oz to 85 g of Selected Fish Cooked Over Dry Heat

Fish	*EPA (mg)*	*DHA (mg)*	*EPA + DHA (mg)*	*Total fat (g)*	*% EPA + DHA of total fat*
Kippered Herring	825	1003	1828	10.5	17
Salmon Atlantic, farmed	587	1238	1825	10.5	17
Pacific and Jack Mackerel	538	1106	1644	7.42	22
Salmon Atlantic, wild	349	1,215	1564	6.9	23
Sardines (Pacific, canned in tomato sauce, drained)	473	824	1297	8.6	15
Tuna Bluefin	309	910	1219	5.3	23
Pickled Herring	711	464	1175	15.3	8
Salmon Coho farmed	347	740	1087	6.4	17
Atlantic Mackeral	428	594	1022	15.1	7
Salmon Sockeye, canned, drained	418	564	982	6.2	16
Trout Rainbow, farmed	284	697	981	6.1	16
Salmon Coho, wild	341	559	900	3.1	29
Trout Rainbow, wild	398	442	840	4.9	17
Sardines (Atlantic, canned in oil,	402	432	834	9.1	9
Bass (Striped)	184	637	821	2.5	33
Trout Mixed species	220	575	795	7.2	11
Tilefish	146	623	769	4	19
Tuna White, canned in water, drained	198	535	733	2.5	29
Swordfish	111	579	690	4.4	16
Pacific, raw (about 1.5)	312	212	524	2	26
Salmon Coho, farmed	347	140	487	1	49
Oysters Eastern, wild, raw (about 6)	228	248	476	2.1	23
Oysters Eastern, wild (about 12)	221	247	468	1.6	29
Whiting	241	200	441	1.4	32
Flounder & sole	207	219	426	1.3	33
Walleye Pollock	157	255	412	0.8	52
Halibut	77	318	395	2.5	16
Rockfish	154	225	379	1.3	29
Alaska king Crab (about 2/3 leg)	251	100	351	1.3	27
King Mackeral	148	193	341	2.2	16
Dungeness Crab (about 3/4 crab)	239	96	335	1.1	30
Oysters Eastern, farmed (about 9)	195	119	314	1.8	17
Tuna Skipjack	77	201	278	0.91	31
Oysters Eastern, farmed, raw (about 6)	160	113	273	1.3	21
Shrimp (about 16 large)	145	122	267	0.9	30
Clams (about 9 small)	117	124	241	1.1	22
Pacific Cod	88	151	239	0.44	54
Tuna Light, canned in water, drained	40	198	238	0.62	38
Tuna Yellowfin	40	191	231	1	23

(Continued)

Table 1 *(Continued)*

Fish	*EPA (mg)*	*DHA (mg)*	*EPA + DHA (mg)*	*Total Fat (g)*	*% EPA + DHA of Total Fat*
Haddock	65	138	203	0.8	25
Wild Catfish	85	116	201	2.4	8
Farmed Catfish	42	109	151	6.8	2
Atlantic Cod	3	131	134	0.8	17
Mahimahi (dolphinfish)	22	96	118	0.8	15
Perch (Atlantic)	88	25	113	1.41	8
Tuna Light, canned in oil, drained	23	86	109	1	11
ScallopsRaw (about 6 large)	16	92	108	0.7	15
Scallops Breaded & fried (about 6 large)	13	88	101	9.3	1
Lobster (Northern)	45	26	71	0.5	14
Atlantic Pollock	7.7	40	48	0.8	6
"Frozen Fish sticks" *(3)*	9	15.6	25	10.3	0

Reprinted from the US Department of Agriculture's National Nutrient Database for Standard Reference, Release 18. Available at *http://www.ars.usda.gov/Services/docs.htm?docid=8964*. <

EPA content is listed in this database as 20:5 n-3 and DHA as 22:6,5 n-3.

presence of Δ-6-desaturase before they are can be fully integrated into biological membranes, while EPA and DHA require very minimal processing.

The overall conversion rate of ALA has been estimated at ≈ 5 to 10% to EPA, and the conversion to DHA is often severely restricted at ≈ 1 to 5% *(12,13,15,16)*. A diet high in LA can restrict this conversion even further, reducing it by 40 to 50% *(12,15)*. This limited conversion is thought to result from the competition of LA and ALA for Δ-6-desaturase which ultimately may limit the rate of either reaction. In fact researchers have shown that while maintaining a constant dietary LA level, 3.7 g of ALA has the same biological effects as .3 g of long chain ω-3 fatty acids, with an overall conversion of 11g ALA to 1 g long chain ω-3 fatty acids *(17)*. To confound the issue even more, there appears to be a differential rate of conversion between women and men. A recent study *(18)* showed that in young (age 28) women the rate of conversion of ALA to DHA and EPA was 2.5 to 200 times greater than the rate in men of the same age, possibly resulting from the action of estrogen *(13,19)*.

Whereas the Institute of Medicine has reported that "ALA is not known to have any specific functions other than to serve as a precursor for synthesis of EPA and DHA," *(20)* ALA has been shown to offer some cardiovascular benefit via its inhibitory effects on platelet clotting and their response to thrombin *(21,22)*, as well as a slight lowering of blood pressure *(23)*. In a prospective study, ALA consumption was shown to be inversely related to the risk of CHD in men *(24)*. The most significant evidence for the use of ALA came from the Lyons Diet heart study, where ALA supplementation in patients recovering from a myocardial infarction resulted in a 35% increase in EPA levels (expressed as % of total fatty acids) and a subsequent 65% relative risk reduction in cardiac death *(25)*.

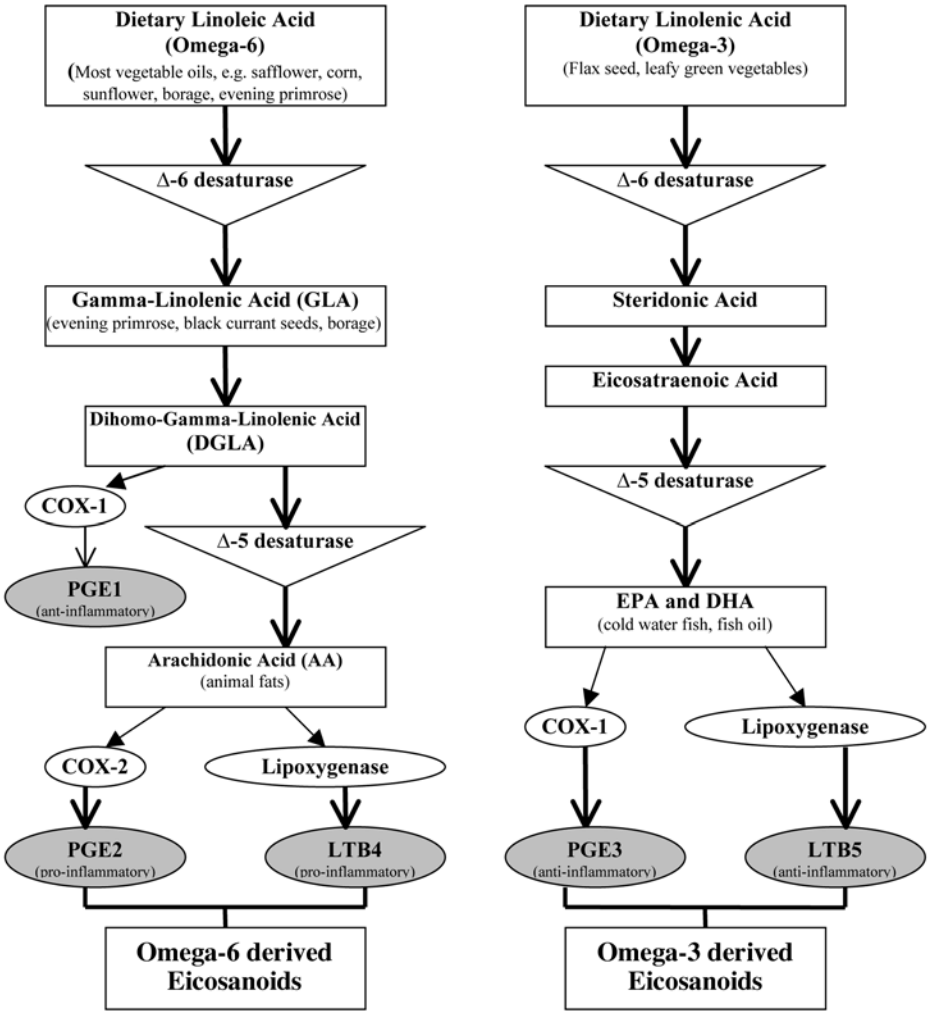

Fig. 1. Dietary fatty acid metabolism and synthesis of eicosanoids from ω-6 and ω-3 fatty acids.

When compared with the ω-3 fatty acids found in fish oil, ALA does not appear to have the same therapeutic capacity. Additionally, ALA is generally not as rapid in its biological effects, which mainly results from its protracted and inefficient conversion to EPA and DHA. Whereas fish oil on the other hand is rapidly incorporated into plasma and membrane lipids and produces almost instantaneous biological effects. A key point worth noting is that the overwhelming majority of studies conferring positive benefits to the consumption of ω-3 fatty acids refer explicitly to oil derived from fish containing the requisite amounts EPA and DHA and not ALA (which may offer its benefit via its modest conversion to EPA and DHA). In the future ALA consumption may be shown to support similar cardiovascular and health benefits of EPA and DHA, for now though fish oil remains to be the single greatest source (in terms of biological effect and availability) of these health promoting compounds.

4. PRENATAL DEVELOPMENT

The health promoting benefits of ω-3 fatty acids begin early in life, as they are essential components of breast milk and have been shown to be of vital importance for both the developing fetus and the mother during pregnancy. Recognizing this, the United States (US) Food and Drug Administration (FDA) in 2002 approved the supplementation of ω-3 fatty acids in infant formula. Approximately 70% of brain cells that are destined to last the lifespan of a human are developed in utero. Whereas arachidonic acid (20:4n-6) is a dominant essential fatty acid in brain tissue, DHA also makes up a significant portion of the fat cells in the brain and is the predominant ω-3 fatty acid in neural and retinal tissue *(26–29)*. Recent studies have shown that a lack of ω-3 fatty acids results in diminished neural development, alterations in the behavior of neurotransmitters, and reduced visual function *(29,30)*. In fact, essential ω-3 fatty acids are required to be transferred from the mother to the developing fetus, and accumulate rapidly—increasing three to five times in the fetal central nervous system during the last intrauterine trimester, the period in which most of the fetal development of the brain, eye, and nervous system occur. If a mother does not maintain a diet rich in ω-3 fatty acids, maternal DHA stores are transferred to meet the demands of, and support the developing fetus *(31,32)*. It is well recognized that increasing maternal dietary intake of DHA during pregnancy will increase both maternal and newborn DHA levels *(33–35)*.

During pregnancy, ω-3 fatty acid supplementation has shown benefit in prolonging gestation and preventing preterm labor. In a randomized double-blind controlled trial, duration of gestation was shown to increase significantly (6.0–2.3 d) when DHA intake was increased to 133 mg daily during the last trimester of pregnancy *(36)*. Similarly, a multicenter study also supported the use of fish oil for prolonging gestation in women at high risk for preterm delivery *(37)*. Women who were supplemented with 2.7 g of fish oil daily reduced preterm delivery rates dramatically, increasing pregnancy duration by 8.5 d and increasing mean birth weight by 209 g compared with a control group. Additionally ω-3 fatty acids may play a positive role in preventing miscarriages. A pilot study in women with persistent antiphospholipid syndrome which is associated with recurrent miscarriages, were supplemented with fish oil over a 3-yr period and found 86% of the women had well babies *(38)*.

5. CHILDHOOD DEVELOPMENT

Given that DHA is highly concentrated in the brain and retina and subsequently plays a vital role in visual and cognitive development, several studies have shown a beneficial correlation between maternal ω-3 supplementation and the cognitive and visual development of infants. In an epidemiological study of 7421 British children, maternal and postnatal fish intake was associated with higher mean development scores at 15 mo of age *(39)*. DHA and AA supplementation was associated with a mean increase of 7 points on the Mental Development Index (MDI) of the Bayley Scales of Infant Development (BSID-II) in a study of 56 children from 5 d to 17 wk of age who received formula supplemented with .35% DHA or with .36% DHA + .72% AA or control *(40)*. Both the cognitive and motor subscales of the MDI showed a significant advantage for children supplemented with DHA and DHA + AA over controls, which suggests that DHA is a major dietary determinant of improved mental development *(40)*. DHA

supplementation has also proven to benefit the development of visual acuity in infants. In a study of 61 healthy breastfed infants who were weaned at 4 to 6 mo to DHA and AA supplemented formula, visual acuity was shown to be significantly more mature in supplemented infants. In addition DHA was more concentrated in red blood cells in the supplemented infants and was associated with greater visual acuity suggesting that the first year of life may serve as a critical period in which a dietary supply of DHA can optimize visual development in infants *(41)*. Contrary to initial expectations which presumed the effects of DHA intake would be immediately noticeable, a recent study indicated that in infants, the benefits of DHA intake during periods of rapid neural development may not be measurable until much later in life. It was found that in the babies of breastfeeding mothers who supplemented with 200 mg/d DHA for 4 mo after delivery, neurodevelopmental indexes and visual function at 4, 8, and 12 mo of age were virtually unaffected. Whereas, at 30 mo of age, the Bayley Psychomotor Development Index was higher in those supplemented with DHA *(42)*. Numerous studies have indicated that maternal dietary and infant formula supplementation with ω-3 fatty acids during pregnancy and lactation can be favorable for later mental development and positively augment a child's IQ later in life *(43–46)*.

6. NEUROPSYCHIATRIC HEALTH

Several studies have shown a positive benefit for ω-3 fatty acids in promoting neuropsychiatric health, as observational studies provided evidence to support the premise that an increased incidence of depression may result from a decreased consumption of fish and ω-3 fatty acids. In the past century the incidence of depression has increased significantly while its age of onset has decreased. In the same time period ω-3 fatty acid intake among Western countries has decreased considerably, with ω-6 fatty acids outnumbering ω-3 fatty acids by a ratio of 20:1. The ideal ratio as reported by several researchers is approx 3:1. Several authors have also shown that cultures consuming large amounts of fish and ω-3 fatty acids tend to have much lower incidences of depression and bipolar disorder *(47–49)*. As can be seen in Fig. 2, those countries consuming the most fish experience markedly lower incidences of major depression than those countries consuming little seafood *(47)*. In fact, some studies have suggested that ω-3 fatty acid consumption may be useful in stemming the depression associated with disease diagnoses *(50,51)*. The positive effects of ω-3 fatty acids in neurobehavioral health and development are not surprising given the high concentration of these EFAs in the brain and their critical role in supporting the body's neural circuitry. Whereas these studies do not ultimately prove that decreased ω-3 fatty acid consumption causes depression, they do provide significant evidence for the role of ω-3 fatty acids in mental health.

Beyond epidemiological data, decreased ω-3 fatty acid levels in erythrocyte membranes of depressed individuals or patients at risk for a recurrent form of major depression have provided significant clinical evidence of the correlation between ω-3 fatty acids and depressive disorders. Several studies have revealed that depressed patients have decreased ω-3 fatty acids in their blood *(52–55)*. There is even a negative correlation between the EPA content of erythrocyte phospholipids and depression severity in some patients, whereas there exists a positive correlation between the ratio ω-6 fatty acid AA to EPA and clinical symptoms of depression *(56)*.

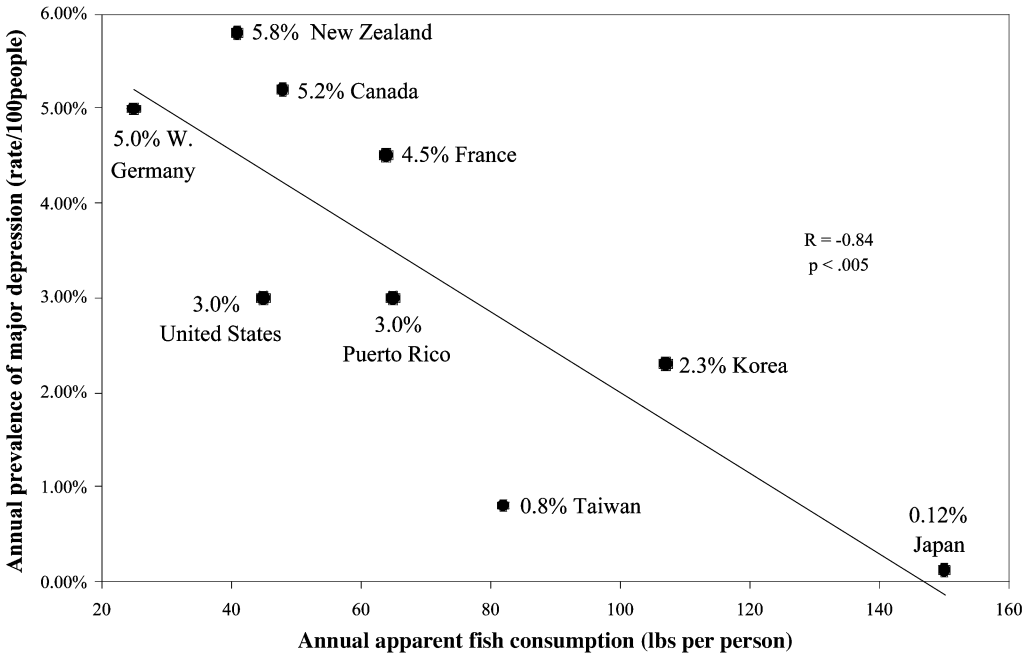

Fig. 2. Fish consumption and major depression—annual prevalence by country simple correlational model with Pearson product moment analysis indicates a potentially substantial interaction between the nearly 60-fold range in annual prevalence rates of major depression and the over 100-fold range of apparent fish consumption, in a multinational comparison (Reprinted with permission from Joseph Hibbeln. Copyright held by the United States Government) *(47)* (1 lb = .4356 kg).

The majority of clinical data and studies investigating the effect of ω-3 fatty acid supplementation on clinical symptoms of depression have been positive, though some smaller studies have shown ω-3 fatty acids to have no effect. In one such study, DHA monotherapy failed to significantly affect the response in patients with major depressive disorder compared with placebo *(57)*. Perhaps the most notable clinical study to date is a 12-wk double-blind, placebo controlled trial in which persistently depressed pretreated patients receiving 1 g of ethyl-EPA daily displayed significantly improved symptoms, with 53% of patients achieving a 50% reduction in the Hamilton depression scale *(58)*. This study showed that a 1 g dose of EPA/d could lead to noticeable improvements in anxiety, depressive moods, sleep patterns, libido, lassitude, and suicidality. Three other studies investigating ω-3 fatty acid supplementation for treatment of depression have shown improvements in scores on depressive rating scales, provided symptom remission and indicated that supplementation was generally well tolerated *(59–61)*. Similar results were seen in treatment of bipolar disorder where researchers determined that patients supplementing with 9.6 g/d ω-3 fatty acids for 4 mo experienced improved outcomes and longer remission compared with placebo *(62)*. The dose of 9.6 g/d fish oil is considered high, though it was well tolerated in this study, other researchers have reported adverse effects such as gastrointestinal upset, worsening glycemia and a rise in LDL-cholesterol for such a high daily dose *(63)*.

Epidemiological data is supportive of ω-3 fatty acid consumption and depression occurrence. Additionally, neurobiological research indicates that ω-3 fatty acids are

critical for proper brain and nervous system function. Though current clinical data is somewhat limited regarding the actual treatment of depression with ω-3 fatty acids, the reviewed studies do show promising results. It would be of great benefit for future studies to test larger patient populations for longer treatment durations to aid in resolving some fundamental treatment principles. For example, it is not clear which fraction (DHA/EPA) or combination of ω-3 fatty acid is most clinically active in depression. Furthermore, a clinically appropriate dose has yet to be established, though fish oil supplementation has been well tolerated and has a consistent long-term safety profile at dose of 1 g/d *(64,65)*.

7. DEMENTIA AND ALZHEIMER'S DISEASE

ω-3 fatty acids have also been shown to have an impact on the onset of Alzheimer's disease and dementia. In several laboratory studies, animals that consumed high amounts of dietary ω-3 fatty acids showed increased concentrations of neurotransmitters, improved control of synaptic activity, and overall protection against oxidation and loss of postsynaptic proteins, all of which have been implicated in the development of Alzheimer's disease *(66–68)*.

Various observational studies have presented convincing epidemiological evidence for the role of ω-3 fatty acids in Alzheimer's disease, suggesting that high fish and/or ω-3 fatty acid consumption is inversely related with cognitive decline, and development of dementia and Alzheimer's disease *(69–73)*. In one retrospective study, the plasma phospholipid ω-3 fatty acid and DHA levels in patients with Alzheimer's disease were 60 to 70% less than the concentrations of controls *(74)*. Perhaps the most compelling evidence for the potential impact of ω-3 fatty acid consumption on the risk of Alzheimer's disease was recently reported in a study of 815 individuals with ages ranging from 65 to 94 yr of age. Researchers found that individuals who consumed fish only once per week reduced their risk of being diagnosed with Alzheimer's disease by 60% when compared with individuals who consumed fish less often *(75)*. This evidence is not surprising considering the fact that synapses contain a high concentration of DHA and ω-3 fatty acids play important roles in the structural and functional maintenance of neuronal membranes and neurotransmission. In fact DHA is the prevalent fatty acid in cerebral gray matter phospholipids and plays an important role in neuronal signaling *(76)*.

There have only been three studies which actually attempted to treat Alzheimer's disease and dementia with ω-3 fatty acid supplementation. In all three studies, EPA and DHA supplementation was shown to increase scores on dementia rating scales, and improve both intelligence and visual acuity *(77–79)*. Doses conferring benefit ranged from 720 mg/d DHA to 900 mg/d EPA. The role for fish oil and its ω-3 fatty acid components may prove invaluable in years to come as the incidence of Alzheimer's disease is expected to increase from the current rate of 12.4% to approx 20% in individuals 65 yr and older by the year 2030 *(80)*.

8. DIABETES

In several epidemiological studies of native populations, lower prevalence of type-2 diabetes has been attributed to diets rich in ω-3 fatty acids. For example, Alaskans and

other Arctic native peoples who consume large amounts of fatty cold water fish exhibit far lower incidences of type-2 diabetes than US and Danish residents who naturally consume less seafood *(81–83)*. In fact, researchers have discovered that in the past 15 yr type-2 diabetes has increased 80% among Alaskan natives, which can be attributed to their increased consumption of nonindigenous foods leading to a fatty acid imbalance *(84–86)*. Japanese Islanders have also been shown to have a lower prevalence of type-2 diabetes than their mainland counterparts, attributed mainly to their dietary consumption of marine foods rich in ω-3 fatty acids *(87)*. Though fish oil has not been shown to clinically impact glycemic control, an extensive review of the literature indicates that fish oil is able to reduce triglyceride levels and decrease the cardiovascular risk associated with type-2 diabetes *(88)*, with a tendency toward significant reduction of low-density lipoprotein (LDL) cholesterolemia *(89)*. As reported in several reviews, patients with type-2 diabetes who consume ω-3 fatty acids may be able to impede disease progression, moderately reduce hypertension, improve blood lipid profiles, and ultimately improve their cardiovascular heath and avoid future cardiovascular complications *(90–93)*.

Though epidemiological data indicate an inverse correlation between seafood consumption and incidence of type-2 diabetes, controlled prospective studies are currently lacking and would provide valuable information that could greatly impact public health. In general, individuals with type-2 diabetes can decrease their risk of CVD without impacting glucose or insulin control by following the recommendations of the American Diabetes Association and the American Heart Association by consuming moderate amounts of fish and ω-3 fatty acids.

9. ARTHRITIS

There is considerable evidence from human and in-vitro studies implicating ω-3 fatty acids as therapeutic agents for the management of inflammatory arthritis. Though the biochemical effects of ω-3 fatty acids in arthritis have yet to be fully elucidated, it is thought that they exert their benefit though the modulation of inflammatory cytokine production. There are over 15 published clinical trials and 2 meta-analyses that support the use of fish oil in treating arthritis *(94,95)*. In addition, these studies have also reported that reducing dietary consumption of ω-6 fatty acids while concurrently increasing ω-3 fatty acid intake can reduce the inflammatory mediators of rheumatoid arthritis and allow certain patients to reduce or suspend the use of nonsteroidal anti-inflammatory drugs (NSAID)s.

Because of the concerns regarding overuse of NSAIDs and their detrimental side effects, fish oil has been emerging as a viable alternative for use in the clinic. In a number of studies, fish oil has shown a modest benefit over NSAIDs. A double-blind placebo controlled trial showed that a dose of 130 mg/kg/d of fish oil decreased the number of tender joints, and duration of morning stiffness, as well as decreased pain and global arthritis activity as compared with placebo *(96)*. In patients with rheumatoid arthritis, fish oil supplementation of 2.8 g/d significantly reduced the need for NSAID use compared with placebo *(97)*. In a 12-mo double-blind randomized trial, 90 patients were supplemented daily with either fish oil or olive oil for 12 mo *(98)*. Fish oil at a dose of 2.6 g/d was shown to exhibit significant clinical benefit, as patients reported much less incidence of pain and experienced a decreased need for anti-rheumatic medication.

10. CANCER

In numerous animal studies, dietary consumption of ω-3 fatty acids has been shown to increase the efficacy of chemotherapy while reducing its side effects, as well as slow the growth of several different cancer xenografts *(99–103)*. Additional support has also come from several epidemiological studies which indicate that populations consuming large amounts of ω-3 fatty acids have much lower incidences of breast, prostate, and colon cancers than those consuming less ω-3 fatty acids *(104–106)*. Generally, ω-3 fatty acids have been show to have anticarcinogenic effects whereas ω-6 and saturated fats have been shown to promote cancer development *(106,107)*. However, recent research suggests that past epidemiological studies have yielded inconsistent results, casting doubt on the significance of ω-3 fatty acid consumption and cancer prevention and/or treatment *(108)*. In one such study researchers investigated the correlation between ω-3 fatty acid consumption and colorectal cancer incidence in a large population of Japanese natives whose diets were rich in fish derived ω-3 fatty acids. The researchers concluded that the findings did not support increasing the consumption of fish derived ω-3 fatty acids in lessening the risk of colorectal cancer *(108)*.

Recently, the authors of a comprehensive systematic review concluded that there is not a significant association between ω-3 fatty acid consumption and cancer incidence *(109)*. Researchers reviewed 38 previous studies of human populations and estimated the effect of ω-3 fatty acids on cancer risk, across 20 cohorts from 7 countries for 11 different types of cancer using 6 different characterizations of ω-3 fatty acid exposure (i.e., supplementation, dietary intake, etc.). For colorectal cancer they estimated 1 study indicated increased risk, whereas 17 studies were without association. For breast cancer they estimated 1 study showed significantly increased risk, 3 were for decreased, and 7 others were without association. For lung cancer they estimated that 1 study was for increased risk whereas 1 indicated decreased risk, and 4 other studies were insignificant. They estimated 1 study for decreased risk in advanced prostate cancer and 1 study for increased risk, whereas 15 other studies did not show any significant association. They were also unable to determine any significant association between ω-3 fatty acid consumption and incidences of cancers of the aerodigestive system, bladder, pancreas, stomach, and ovaries, as well as lymphoma.

Perhaps fish oil's most applicable use in oncology may be in its treatment of cancer related cachexia. Cachexia represents an abnormal change in satiety, evident by a marked reduction in food intake which can negatively impact prognosis in some cancer patients *(110,111)*. Cachexia is a common complication in cancer patients, with those suffering from gastric and pancreatic cancers having the highest frequency (83–97%) *(112)*. Inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1 (IL-1), and interferon-β (IFN-β) produced by activated monocytes/macrophages are thought to be responsible for signs and symptoms of cahcexia *(113)*. Fish oil has been shown to cause decreases in the production of the inflammatory cytokines IL-6, TNF-α, and IL-1 in healthy adults *(114)*, and high doses of EPA have lead to reductions in serum C-reactive protein levels, also an inflammatory marker *(115)*. Fish oil supplementation is believed to be able to mediate the effects of inflammatory cachexia through the down-regulation of proinflammatory cytokine release. In fact, results from open-label studies have indicated that fish oil may reverse cachexia in patients with advanced pancreatic cancer *(115)*. Though the effects of fish oil supplementation for the treatment of cancer cachexia appear initially promising,

further results from future clinical trials are necessary before a therapeutic recommendation can be made.

11. MECHANISMS OF ACTION

There are a vast number of studies supporting the biological activity of ω-3 fatty acids and multiple mechanisms of action have been proposed. In events such as fetal and childhood development in which ω-3 fatty acids are vital components of neural and retinal cells, their activity may be likened to the effect of amino acids as fundamental cellular building blocks. Additionally the presence of DHA in biological membranes appears to play an important role in homeostasis and cell signaling, an example is DHA's ability to maintain levels of cellular phosphatidlyserine *(116)*. In specific instances ω-3 fatty acids have been shown to improve cardiac function by preventing intracellular calcium overload and reducing the concentration of markers such as creatine kinase *(117)*. This activity may be due to the ability of DHA and EPA to prolong the refractory state of cardiac myocytes by interacting with fast acting sodium channels and L-type calcium channels *(118,119)*. EPA and DHA have also been shown to reduce fasting and postprandial triglyceride levels by as much as 35% via acceleration of chylomicron triglyceride clearance and by suppressing VLDL-triglyceride production *(120)*.

Much of the current literature suggests that the tremendous health promoting activity of ω-3 fatty acids may be attributed in large part to their anti-inflammatory properties. EPA, DHA and arachidonic acid (AA) are all precursors for the formation of thromboxane (Tx), prostaglandins (PGs), and leukotrienes (LTs), all of which belong to the eicosanoid family of compounds. Eicosanoids are localized hormones created in cells and are known to function as regulators of blood clotting factors and cellular calcium channels, active in dilation and contraction of muscles, regulators of hormone and digestive secretions, they also control fertility, growth, and cell division *(121)*. Depending on which fatty acid the eicosanoid is derived from can greatly influence its biological activity as can be seen in Fig. 1. Generally, AA derived compounds tend to be highly proinflammatory and can increase vasoconstriction, promote thrombosis, and increase pain. Conversely EPA and DHA tend to promote an anti-inflammatory response by producing a different class of eicosanoids which are able to inhibit the activity of some of AAs proinflammatory compounds. Additionally EPA and DHA are effectively able to displace and reduce AA incorporation into cellular membranes, ultimately promoting an anti-inflammatory response.

12. INTAKE RECOMMENDATIONS

The current average intake of ω-3 fatty acids in the US is approx 1.6 g/d, including .1 to .2 g/d as EPA and DHA *(63)*. Several expert panels and organizations have made recommendations for the consumption of ω-3 fatty acids and are summarized in Table 2 *(20,32,63)*. The Institute of Medicine in collaboration with Health Canada recommends consuming 1.3 to 2.7 g/d ω-3 fatty acids for an average 2000 calorie diet. The World Health Organization (WHO) recommends consuming .4 to 1 g/d of EPA and DHA. Both of these recommendations can be met by consuming two servings of fish/wk *(63)*. Since the year 2000, the American Heart Association (AHA) has recommended that healthy adults consume at least two servings of fish/wk, especially cold-water oily fish rich in DHA and EPA. The AHA also recommends .5 to 1.8 g/d of EPA and DHA to

Table 2
Summary of Recommendations for ω-3 Fatty Acid Intake

International Society for the Study of Fatty Acids and Lipids (ISSFAL) *(32)*	Recommends .3% of daily energy from EPA + DHA. Minimum of 300 mg/d of DHA for pregnant and lactating women. Minimum of 500 mg/d EPA for Cardiovascular Health.
Institute of Medicine's Dietary Reference Intakes 2002/2005 *(20)*	Recommends .6 to 1.2% of energy from ω-3 fatty acids (1.3–2.7 g/d based on 2000 calorie diet)
USDA Dietary Guidelines 2005	Recommends 8 oz/wk (2 servings) of fish high in EPA and DHA.
WHO 2003 *(63)*	Recommends 1 to 2 servings of fish/wk for total of 400 to 1000 mg EPA and DHA.
NIH sponsored expert panel, 1999 *(32)*	Recommends 300 mg/d DHA for pregnant and lactating females.
American Heart Association, 2002 *(63)*	
Patients without documented CHD	Eat a variety of (preferably fatty) fish at least twice/wk. Include oils and foods rich in α-linolenic acid (flaxseed, canola and soybean oils, flaxseed and walnuts).
Patients with documented CHD	Consume about 1 g of EPA + DHA/d, preferably from fatty fish. EPA + DHA supplements could be considered in consultation with the physician.
Patients who need to lower triglycerides	2 to 4 g of EPA + DHA/d provided as capsules under a physician's care.

reduce cardiac disease. A word of caution concerning potential contaminants should be advised when attempting to meet the recommendations of consuming two servings of fish/wk. This concern is especially relevant for pregnant and nursing mothers as well as young children, as undue levels of mercury can harm fetal development and damage the nervous system of young children. The US Environmental Protection Agency (EPA) and FDA recommends that women and young children avoid eating shark, king mackerel, tilefish, and swordfish, and that no more than an average of 2 servings (12 ounces total) of low-mercury fish be eaten per week. Table 3 shows the most commonly eaten fish and their mercury content. Five of the most commonly eaten fish that are lowest in mercury are canned light tuna, shrimp, salmon, pollock, and catfish, note that albacore tuna has much more mercury than "chunk light" and that consuming no more than 6 ounces of albacore/wk is recommend by the EPA and FDA.

13. CONCLUSION

Current research clearly shows that fish oil is the best known dietary source of ω-3 fatty acids. This research also confirms that ω-3 fatty acids are fundamental components of healthy biological systems, and their depletion can often lead to potentially preventable health complications. Research has suggested that the correction of subtle nutritional deficiencies such as low dietary intake of ω-3 fatty acids may reduce the risk of

Table 3
Mercury Content of Popular Fish

Species	*Mercury (PPM)*
Tilefish (Gulf of Mexico)	1.45
Shark	0.99
Swordfish	0.97
Mackerel King	0.73
Grouper	0.55
Orange Roughy	0.54
Marlin	0.49
Tuna (Fresh/Frozen)	0.38
Tuna (Canned, Albacore)	0.35
Bluefish	0.31
Lobster (American)	0.31
Croaker White (Pacific)	0.29
Scorpionfish	0.29
Bass (Saltwater)1	0.27
Halibut	0.26
Weakfish (Sea Trout)	0.25
Sablefish	0.22
Buffalofish	0.19
Snapper	0.19
Monkfish	0.18
Tilefish (Atlantic)	0.15
Carp	0.14
Perch (Freshwater)	0.14
Skate	0.14
Sheepshead	0.13
Tuna (Canned, Light)	0.12
Cod	0.11
Squid	0.07
Whitefish	0.07
Crab	0.06
Pollock	0.06
Catfish	0.05
Scallops	0.05
Anchovies	0.04
Herring	0.04
Haddock	0.03
Trout (Freshwater)	0.03

(Continued)

future chronic diseases. Some authors have even likened this approach to the treatment of scurvy with vitamin c, osteoporosis with vitamin D and calcium, or the prevention of neural tube defects and anemia by supplementing folic acid and other B-vitamins *(122)*. Components of fish oil such as EPA and DHA are EFAs that are able to alter cellular

Table 3 ***(Continued)***

Species	*Mercury (PPM)*[a]
Sardine	0.02
Salmon	0.01
Hake	0.01
Tilapia	0.01

Adapted from the US EPA "Advisory of Mercury in Seafood." Available at *http://www.cfsan.fda.gov/%7Efrf/sea-mehg.html.*

Note: Source of data: FDA Surveys 1990–2003:

"National Marine Fisheries Service Survey of Trace Elements in the Fishery Resource" Report 1978; "The Occurrence of Mercury in the Fishery Resources of the Gulf of Mexico" Report 2000;

Market share calculation based on 2001 National Marine Fisheries Service published landings data.

[a]Mercury was measured as total mercury and/or methylmercury.

membrane composition and are vital components of neural cells. Their availability to the developing fetus appears to impact the future retinal and neural health of the newborn, and they also seem to significantly affect pregnancy duration. ω-3 fatty acids are able to modulate inflammatory cytokine and eicosanoid production thereby affecting the activity and abundance of key transcription factors and inflammatory mediators. Several clinical and epidemiological studies provide great support for the beneficial effects of ω-3 fatty acids in the prevention and treatment of numerous chronic diseases such as, CVD, rheumatoid arthritis, depression, and Alzheimer's disease. ω-3 fatty acids may also be potentially useful in type-2 diabetes as a means to lessen cardiovascular risk factors. Results for using ω-3 fatty acids in oncology are mixed and at this time they are not believed to have any significant benefit, though their use for cancer related cachexia seems promising. Emphasis should be placed on the dietary essentiality of ω-3 fatty acids and the simple fact that therapeutic doses can be safely obtained from moderate intake of cold water fatty fish. Overall, the diverse impact of ω-3 fatty acids on health promotion is currently positive and those wishing to experience such benefits are encouraged to adopt a healthy dietary approach maximizing ω-3 fatty acid intake early and throughout their lives.

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16 Omega-3 Fatty Acids and Mediterranean Diet in the Prevention and Treatment of Cardiovascular Diseases

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Abstract

Mediterranean diet and ω-3 fatty acids are the two dietary strategies that were unambiguously shown to be effective in reducing the complications of coronary heart disease (CHD) in randomized controlled trials. Regarding the ω-3 issue, this raises two questions: (i) Which are the dietary factors in a Mediterranean diet that are particularly protective against CHD? and (ii) are ω-3 fatty acids mediators of the protective effect resulting from the adoption of a traditional Mediterranean diet?

One of the basic conclusions drawn from main nutrition clinical studies and prevention trials is that low-fat diets are not protective against CHD. In contrast, diets including a low saturated fat intake (whatever the total fat intake) were shown to reduce the risk of CHD complications, but only if they are not associated with an increased consumption of polyunsaturated fatty acids (PUFA). This is in total agreement with the concept of Mediterranean diet, a dietary pattern associated with high protection against CHD and characterized by low consumption of both saturated fats and PUFA.

Epidemiological studies and controlled trials indicate that plant ω-3 fatty acids likely are important mediators of the protection provided by the traditional Mediterranean diets. Regarding marine ω-3 fatty acids, the consumption of fish is very high at present in certain Mediterranean countries, such as Spain and Portugal, whereas it is quite low in others (Italy, Greece). This suggests that the relative deficiency in marine ω-3 fatty acids among Italians might explain the surprising results of the GISSI Prevenzione trial, in which a small supplementation in EPA + DHA (about 800 mg/d) in secondary prevention resulted in a striking reduction of the risk of CHD death (30% reduction) and of sudden cardiac death (45% reduction). If this hypothesis is valid, it also suggests that the protection provided by omega-3 fatty acids is partly dependent from other associated dietary factors.

We conclude that plant and marine ω-3 fatty acids are major mediators of the protective effect provided by the traditional Mediterranean diets.

Key Words: Mediterranean diet; ω-3 fatty acids; coronary heart disease.

1. INTRODUCTION

Mediterranean diet and omega-3 fatty acids are the two dietary strategies that were unambiguously shown to be effective in reducing the complications of coronary heart disease (CHD) in randomized controlled trials *(1–5)*. However, this raises two important questions:

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Table 1
Main Characteristics of the Traditional Mediterranean Diet

1.	High diversity and seasonality.
2.	Predominance of vegetables and fruits.
3.	Importance of whole cereals (especially bread and couscous) and pulses.
4.	Olive oil almost the sole edible oil.
5.	Cheese and yogurt in moderation.
6.	Fish and seafood in moderation.
7.	Large use of garlic, onion, herbs and spices.
8.	Wine in moderation, or tea.

(i) which are the dietary factors in the Mediterranean diet that are particularly protective against CHD? and (ii) are ω-3 fatty acids mediators of the protective effect resulting from the adoption of a traditional Mediterranean diet?

Epidemiological studies and controlled trials have actually shown that adherence to a Mediterranean diet pattern is associated with (or results in) a lower CHD mortality rate and improved survival *(1,2,6–8)*. Before discussing which Mediterranean dietary factors are protective and whether ω-3 fatty acids are major players in this context, we first have to define the Mediterranean diet.

2. DEFINITION OF THE MEDITERRANEAN DIET

There are several "Mediterranean" diet patterns around the Mediterranean Sea. Their definition varies according to the nationality of the experts and to the geographic location of the populations studied. For instance, the traditional diet in Southern Italy is different from that in Northern Africa, and the diet of Catalonia in Spain is obviously different from that of Lebanon in the Eastern Mediterranean area. However, there are also strong similarities between the dietary habits of these populations, such as a large consumption of cereals (essentially wheat) and an extremely high intake of polyphenol-rich beverages, although it is under the form of wine in the North of the Mediterranean Basin, and tea in the South.

Ecological comparisons of different cohorts in the Mediterranean area during the 1960s and 1970s (before fast-food restaurants started spreading over the area) showed that the Greeks, especially those living in the islands of Crete and Corfu, had the lowest mortality rate from CHD among Mediterranean populations *(9)*. Thus, it seemed that if a Mediterranean diet pattern was to be selected among several others to provide a preventive strategy, it would be preferable to focus on the dietary habits in these Greek islands *(9)*. The investigators of the Lyon Diet Heart Study did so and, as expected, they were successful *(1,2)*. It is nonetheless possible to define the Mediterranean diet in general through some major characteristics (Table 1).

If we then define the traditional Mediterranean diet on the exclusive basis of its lipid characteristics, we can enumerate at least seven "Mediterranean lipid factors" (Table 2). To simplify, it is preferable to use qualitative rather than quantitative terms, and the lipid factors are described by comparison with the same factors in Western Europe and the United States (US).

Table 2
The Traditional Mediterranean Diet in Terms of Lipid Factors

1.	Total fat intake: variable.
2.	Saturated fat intake: low but not very low (about 8–10% of total energy intake).
3.	Monounsaturated fat intake: very high (higher than 13%).
4.	ω-6 polyunsaturated fatty acid intake: very low (less than 10 g/d).
5.	Plant ω-3 polyunsaturated fat intake: high but not very high (at least 2 g/d).
6.	Marine ω-3 polyunsaturated fat intake: variable.
7.	Trans fatty acid intake: very low (possibly none at all).

The next questions are to determine which of these Mediterranean lipid factors are important for the prevention and treatment of CHD complications, and whether ω-3 fatty acids are critical determinants of the Mediterranean diet protection.

3. LESSONS FROM THE SEVEN COUNTRIES STUDY

In the Seven Countries Study, Keys et col. showed that the CHD mortality rate was positively associated with saturated fat intake and inversely associated with monounsaturated fat intake *(9)*. In other words, the more one eats animal fat, the higher the risk of CHD death; the more one eats olive oil, the lower the CHD death risk *(9)*. This was major information about the relations between diet and CHD.

At the same time—and this a second lesson drawn from the Seven Countries Study—it was clear that total fat intake was not a critical factor in the study, because the Japanese cohort, with 25% of energy as total fat, and the Greek cohort, with 40% of energy as fat, had the lowest (and similar) mortality rates from CHD as compared with the other European or non-European cohorts *(9)*. As expected, a recent large randomized trial conducted in postmenopausal women confirmed that a low fat dietary pattern does not significantly reduce the risk of CHD and stroke *(10)*.

The third lesson taught by the Seven Countries Study was that the different Mediterranean cohorts of the study did not have the same risk of CHD death despite apparently similar dietary habits. The risk was higher for ex-Yugoslavians than for Greeks and higher for Italians than for ex-Yugoslavians *(9)*. It is therefore very important to understand what the differences between these populations were when the study was conducted. We discuss the issue about the Italian cohort in the last section of this chapter.

The fourth point regards the consumption of fish and marine ω-3 fatty acids. At present, the consensus among scientists and physicians is that populations who eat a lot of fish and marine foods are relatively protected against CHD death. The typical population with high-fish intake is the Japanese, who have the lowest CHD mortality rate in the world. However, some other high-fish populations are apparently not protected. For instance, the Finns eat much more fish than the Italians or the British (at least 50% more) but their CHD mortality rate was much higher than that of the Italians, and one of the highest in the world. Thus, other factors than fish seem to interplay with fish intake to nullify the potential protective effect of ω-3 fatty acids. This means that the protective effect of ω-3 fatty acids depends on context, life style or specific dietary factors. It would be obviously critical to identify which factors were interacting with ω-3

fatty acids in the Finnish cohort of the Seven Countries Study. Subsequent studies have clearly shown that the Finns were characterized (in addition to the traditional risk factors such as smoking and high cholesterol) by an extraordinary combination of dietary risk factors, including high mercury (from fish), low selenium (a major antioxidant and the antidote to heavy metal toxicity), low vegetable antioxidants (especially carotenoids and polyphenols), and a high saturated fat intake *(11–13)*. It is therefore thinkable that the potential protection owed to fatty fish was neutralized by this constellation of harmful dietary factors.

On the other hand, all these data have been drawn from earlier observational studies (including the Seven Countries Study) and they have to be confirmed by more recent epidemiological studies and (preferably) controlled trials. In fact, randomized trials are the only way to demonstrate a cause-effect relationship. In the next paragraphs, we will particularly discuss the saturated fat and ω-3 fatty acid issues.

4. THE SATURATED FAT INTAKE ISSUE

Is saturated fat intake a critical factor of CHD mortality? After the Seven Countries Study, data from subsequent epidemiological studies were less consistent *(14,15)*, whereas the results of controlled trials were confusing *(16)*. At least four controlled trials testing the hypothesis that reduction of saturated fat may decrease the risk of CHD complications in secondary prevention have been published *(16–18)*. There is no room here to fully discuss each of them. In the Los Angeles trial, for instance, which has often been presented as a landmark successful trial demonstrating the effect of reducing saturated fat intake to prevent CHD complications, the numbers of deaths in the control and experimental groups were 121 and 126 respectively, indicating no effect at all *(17)*. In the London and Sydney trials, there also was no effect on mortality *(16)*. Finally, in the first Oslo trial, there was a small (but transient) borderline significant effect on cardiac mortality *(18)*. Thus, despite a striking reduction in saturated fat intake and a significant effect on serum cholesterol, there was no convincing effect on CHD mortality in these trials *(16)*. Curiously, some scientists still regard them as successful and this kind of strategy as relevant for clinical prevention *(19)*.

The trials designed for primary prevention were also confusing, especially those conducted in mental hospitals in Finland and in the US *(20–22)*. In fact, when carefully looking at the experimental diets in these various trials *(16–18)*, it appears that the intervention was not restricted to the reduction of saturated fat intake. The investigators also asked (and helped) the patients in the experimental group to increase their consumption of polyunsaturated fats (PUFA). As a consequence, the total fat intake (the sum of saturated fat and PUFA) was the same in the two groups of each trial, but the PUFA/saturated fat (P/S) ratio was strikingly increased in the experimental groups of each trial. This strategy did introduce at least three biases to interpret the effect of reducing saturated fat intake. First, a large increase in PUFA may have modulated the effect of reducing saturated fats and nothing can be concluded regarding the effect of the reduction of saturated fats itself. Second, the large intake in PUFA in these trials was essentially the result of an increased intake in ω-6 fatty acids and, even if ω-6 fatty acids are effective to decrease serum cholesterol, they have never been shown to be protective against CHD complications. In addition, no population in the world has ever adhered to such high

ω-6 diets suggesting that ω-6 PUFA have never been recognized as major healthful nutrients beyond the indispensable amounts needed to prevent essential PUFA deficiency. Third, depending of the type of edible vegetable oil (and margarine) used to increase the consumption of PUFA, the increase in ω-6 fatty acids was or was not associated with an increased consumption of plant ω-3 fatty acids. In that case (for instance in the first Oslo trial), and in view of the well-known protective effect of plant ω-3 fatty acids *(1,23–27)*, any protection observed in a trial could be attributed to an increase in ω-3 rather than to an increase in ω-6 PUFA or to a decrease in saturated fat.

Thus, considering the whole data, the only thing we could conclude from most dietary prevention trials is that "a high consumption of ω-6 fatty acids did not appear to be more protective than a high saturated fat intake." This is actually major information. However, we still do not have any answer regarding the specific question of saturated fat. One trial, however, did provide useful data regarding these issues. It is the Second Oslo trial *(28)*, where for the first time in a dietary trial, an unambiguously significant effect on CHD and total mortality was reported. After 102-mo follow-up, there was a 40% reduction of total mortality in the experimental group. In addition, after 60-mo follow-up, the authors also reported a 47% lower incidence of fatal and nonfatal CHD complications. As only 24% of the smokers in the experimental group completely stopped smoking (one of the aims of the trial was to help patients quit smoking) vs 17% in the control group, the striking differences in mortality and CHD complications between groups could not be explained by the difference in smoking *(28)*. In contrast, the diet of the experimental group was very different from that of the control group, mainly because of a reduction of saturated fat, which made up 18% of total energy in the control group and only 8% in the experimental group. The consumption of PUFA was the same in the two groups: 8 and 7% in the experimental and control groups, respectively. As a consequence, it can be assumed that the improved prognosis in the experimental group essentially resulted from the difference in the consumption of saturated fat intake. If we except the Lyon Diet heart Study (where the decrease in saturated fats was associated with a decrease in total PUFA, and increases in monounsaturated fats and ω-3 fatty acids) the second Oslo Trial has been the only trial to date in which the reduction of saturated fat was not associated with an increased consumption of PUFA. Another major lesson drawn from the second Oslo trial is connected to the previous trials: if, as written above, "a high consumption of ω-6 fatty acids is not better than a high saturated fat intake," the finding that a high saturated fat intake is unhealthy automatically implies that a high ω-6 fatty acid intake also may be harmful. This is in line with previous trials including a high ω-6 intake, which were negative *(16–18)*, but in contradiction with epidemiological studies claiming that a high ω-6 fatty acid intake is associated with a decreased risk of CHD complications *(14,15)*. It is noteworthy that in most epidemiological studies reporting negative associations between PUFA intake and CHD complications, the authors did not clearly differentiate ω-3 and ω-6 fatty acids. Thus, they were not able to say whether protection was related to a high ω-3 fatty acid intake (which would not be a surprising finding) or to a high ω-6 fatty acid intake, or both. Our opinion is that when there is discrepancy between data from trials and data from epidemiological studies, it is obviously preferable to trust trial data.

The main conclusion drawn from these trials was that low saturated fat intake does reduce the risk of CHD complications, but only if it is not associated with an increased

consumption of PUFA. This is in total agreement with the concept of Mediterranean diet, a dietary pattern associated with a high protection against CHD and characterized by a low consumption of both saturated fats and PUFA.

5. THE ω-6 POLYUNSATURATED AND MONOUNSATURATED FAT ISSUE

No trial so far testing the effect of a high consumption of ω-6 PUFAs only has shown convincing protective effects against CHD complications. In some cases, such as the Finnish Mental Hospital Studies *(20,21)*, in which the investigators reported a significant effect on mortality, it was not clear whether the effect resulted from an increased consumption of ω-3 fatty acids or to the ω-6 fatty acids. In addition, considering the conditions required to validate and classify CHD complications in the clinical setting (importance of the timing for electrocardiogram [ECG] recording and blood sampling, importance of the description of chest pain by the patients) and the absence of blinding in the studies, hospitalized psychiatric patients with severe mood disorders and heavy drug therapy are certainly not the ideal population to conduct such trials. In addition, as discussed above about the reasons why the Finnish are not protected by their consumption of fish, it appears that the results of trials conducted in Finland cannot be extrapolated to other populations with very different risk profiles. As a matter of fact, the Minnesota Coronary Survey *(22)*, a dietary intervention similar to that tested in the Finnish studies (and also conducted in mental hospitals, but in the US), did not confirm the Finnish data (no difference in mortality between groups, despite a significant effect on serum cholesterol), which suggests that the data from the Finnish Mental Hospital Studies *(20,21)* should be taken with precaution.

Finally, no controlled trials specifically tested the hypothesis that a high monounsaturated fat intake may protect against CHD complications. This was a major component of the Lyon Diet Heart Study *(1,2)*, but in the context of a multifactorial intervention no definite conclusion can be drawn for one particular component of the experiment. On the other hand, a high monounsaturated fat intake and a high consumption of olive oil are among the main characteristics of the Mediterranean diet pattern, which is generally regarded as protective against CHD. Further well-designed trials are required to examine the specific effects of oleic acid independently from the other components of olive oil.

6. THE ω-3 FATTY ACID ISSUE

The ω-3 fatty acid issue in the context of the Mediterranean diet raises two main questions. First, are ω-3 fatty acids among the Mediterranean protective factors? Second, are ω-3 fatty acids traditionally consumed by the Mediterranean populations? We will not fully discuss whether and how ω-3 fatty acids are protective in this chapter, but only whether they are major components of the Mediterranean diet.

When considering ω-3 fatty acids, it is important to distinguish those from plant and from marine sources. The main plant ω-3 fatty acid is α-linolenic acid (ALA), the precursor of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in mammals through pathways involving elongation and desaturation of the carbonyl chain *(23)*. ALA is an essential fatty acid, which means that we are not able to synthesize it and need a daily supply from our diet (at least 2 g/d). EPA and DHA are theoretically not essential but, in fact, our ability to synthesize them from ALA is limited, especially for

the final step of the pathway leading to DHA *(29)*, and it is generally considered that our diet should provide us with minimum amounts of them (about 1 g/d).

Are ω-3 fatty acids traditionally consumed by the Mediterranean populations? Only recently did some data about this question appear in the literature. In 1993, Sandker et al. reported a comparison of the blood fatty acids of participants of the Greek and Dutch cohorts of the Seven Countries Study, showing that the Greeks had three times as much ALA in their blood as the Dutch and an ω-6 linoleic acid (LA) level lower by 20% *(30)*. Because ALA and LA are essential fatty acids, this indicated that the traditional Greek diet is rich in ALA, but poor in LA and ω-6 fatty acids in general. This was not a surprising finding since the edible oil used by the Greeks is almost exclusively olive oil, which is poor in LA and ω-6 fatty acids. Curiously, there was no data regarding EPA and DHA in that report. As olive oil is also poor in ALA, the next question was to identify the sources of ALA in the Greek diet. The main ones, as identified by Simopoulos, include walnuts, eggs from range-fed hens, purslane and some other green leafy vegetables, some meats often consumed by the Greek (rabbit), and cheese and yogurts (provided the animals were fed ALA-rich aliments such as fresh grass and wild plants) *(31)*. Thus, the Mediterranean diet of Crete is rich in plant ω-3 fatty acids, and we can assume that this was probably also the case in other parts of the Mediterranean Basin, when and where industrial agriculture and extensive rearing (using ω-6 rich cereals and foods for animals) have not already invaded the area.

The main lesson from this finding is that any dietary trial based on the concept of Mediterranean diet should include a high consumption of ALA. The investigators of the Lyon Diet Heart Study did so, and they obtained a significant increase in the blood concentration of ALA and significant protection against CHD complications *(1,2)*, which confirmed previously published epidemiological studies *(9)*. Furthermore, when they analyzed their data in a multivariate model to study the relations between various dietary factors and the risk of CHD complications, the first dietary factor to be associated with protection was ALA *(2)*. Thus, plant ω-3 fatty acids seem to be important mediators of the protection provided by the traditional Mediterranean diet. This does not mean, however, that dietary ALA should be considered in isolation (something like a drug approach of prevention) and not in the context of the Mediterranean diet. Rather, we believe that dietary ALA absolutely must be included within a global dietary approach, as one factor interacting with many others, such as those enumerated in the Mediterranean lipid and nonlipid factors of Tables 1 and 2.

Regarding the consumption of marine ω-3 fatty acids (EPA and DHA) by Mediterranean populations, things are quite clear when looking at the Food and Agriculture Organization (FAO) Food Balance Sheets of the World Health Organization (WHO) (Table 3). In certain countries, such as Spain and Portugal, the intake of fish is very high, whereas it is quite low in other countries (e.g., Italy and Greece). However, this is only a crude estimate of the actual intake of marine ω-3 fatty acids, because the concentrations of EPA and DHA vary from one fish species to another (demersal vs pelagic vs freshwater fish). The only way to get a realistic image of EPA + DHA consumption is by using biomarkers, for instance testing EPA and DHA levels in plasma or red cells. Using such parameters in the IMMIDIET study *(32)*, we have found that the concentrations of EPA and DHA were lower among Italians living in Southern Italy than among British or Belgian subjects (data not published). This suggests that at the

Table 3
2002 Fish Supply (kg/yr/capita) in Different Mediterranean and Non-Mediterranean Countries According to FAO Food Balance Sheets

Japan	66
Norway	55
Spain	48
France	31
Italy	26
Greece	23
United Kingdom	23
United States	21

present times, the consumption of marine ω-3 fatty acids by certain Mediterranean populations (i.e., the Italians) may be very low.

Following this observation, an important question is whether the relative deficiency in EPA + DHA among Italians may explain the surprising results of the GISSI Prevenzione trial *(4)*, in which a small supplementation in EPA + DHA (about 800 mg/d) in secondary prevention resulted in a striking reduction of the risk of CHD death (30% reduction) and of sudden cardiac death (45% reduction). At the same time, there was no difference between groups in the risk of stroke and of nonfatal CHD complications (new infarction and unstable angina). It is noteworthy that before randomization, all patients were advised to return to the traditional diet of their parents and grandparents, and that most of them did so *(4)*. If the patients in both groups were following their parents' Mediterranean diet, the absence of difference between groups for nonfatal CHD complications is not surprising. It means that both groups were equally protected because of their diet and that the small dose of EPA + DHA given in the experimental group had no significant effect on the progression of the arterial disease. At the same time, if these patients were relatively deficient in ω-3 fatty acids (with a high risk of ischemia-induced ventricular arrhythmias), it is also not surprising that an EPA + DHA supplementation with dosages as low as 800 mg/d did result in a significant reduction of the risk of CHD death and sudden cardiac death. Thus, the results of the GISSI trial are in line with the concept of Mediterranean diet and with the current nutritional situation in the various countries of the Mediterranean area.

7. SUMMARY AND CONCLUSIONS ABOUT ω–3 FATTY ACIDS

In spite of major advances in the understanding and treatment of CHD in recent years, the causes of the persistent epidemics are still unknown. However, biologists, epidemiologists and clinician cardiologists do agree that ω-3 fatty acids from both marine and terrestrial plant sources are very important in the development (and prevention) of CHD complications.

They are important for at least three main reasons: first, they are indispensable nutrients for the cardiac cells and in case of deficiency (or insufficiency), physiology of cells is not optimal; second, ω-3 fatty acids are said essential which means that humans do not make them by their own (not at all for ALA, in very short amounts only for

docosahexanoic acid or DHA) and need them in their daily meals; third, we have now strong evidence that most populations in the world are relatively deficient in ω-3 fatty acids and that higher the deficit higher the rate of CHD mortality is. To illustrate the point, let's have a brief look at the main sources of ω-3 fatty acids in our modern industrial world and as seen through the statistics of the Food and Agriculture Organization (FAO) of the United Nations (www.fao.org/index_fr.htm).

In 2002, the consumption of fish by the Japanese (the population with the lowest mortality rate from CHD in the world) was in average 67 kg whereas it was 21 kg in the US and 18 kg in Russia, the two countries with highest CHD prevalence. It was 48 kg in Spain and 55 kg in Norway, two countries with "intermediate" mortality rates from CHD. According to the FAO, the consumption of rapeseed oil, which is the best plant source of vegetable ω-3 fatty acids (high bioavailability of ALA when taken with rapeseed oil as opposed to soybean oil for instance), was 6 kg by Japanese, 1.5 kg by Americans, and 0 kg by Russians in 2002. For these reasons, experts from the American Heart Association recently wrote that attaining the proposed recommended ω-3 fatty acid intake will require an approximately fourfold increase in fish consumption and twofold increase in consumption of vegetable ω-3 fatty acids. The same could be said for the UK citizens and even for the French. These recommendations are based not only on epidemiological and ω-3 consumption data but also on medical studies. Biologists and physiologists have indeed clearly shown in various experimental models the importance of ω-3 fatty acids to keep our cardiac cells healthy and able to resist to heart attacks (ischemic damage). Finally, clinicians have conducted randomized trials in thousands of CHD patients and have shown that even small increase in the consumption of ω-3 fatty acids (particularly in the context of a Mediterranean diet) can significantly improve the prognosis after a heart attack. It is noteworthy that in these ω-3 trials, and in contrast with most drug trials, there was a significant effect on survival and life expectancy. For instance, in the Lyon Diet Heart Study (in which Michel de Lorgeril was the first investigator), mortality was reduced by more than 50% over a follow-up of 4 yr.

The next obvious question is: what should we do to increase our consumption of ω-3 fatty acids? Before answering that question, it is important to recall that the effectiveness of ω-3 fatty acids to protect our heart is higher if we adopt a favourable "global" dietary pattern such as the traditional Japanese or Mediterranean diets as written in the previous section of that text. The reason is that there are many interactions between nutrients and that some nutrients can counteract the protective effects of others. The best example is given by soybean oil, the consumption of which by the American is huge, reaching 22 kg/yr/person vs 5 kg by the Japanese, 4 kg by the Spanish, 3 kg by the British, or 1.5 kg by the French). However, in soybean oil (but not in rapeseed oil) ω-3 fatty acids (primarily ALA, the precursor of the very long chain found in fatty fish) are associated with quite high amounts of ω-6 fatty acids. ω-6 fatty acids compete with ω-3 at different levels and alter the metabolism of ALA. The ω-3:ω-6 ratio in soybean oil results in a nonoptimal blood and cellular ω-3 status. This explains why, paradoxically, US consumers do not have in average an adequate ω-3 status despite a huge consumption of soybean oil.

Regarding now the practical question of how to improve our ω-3 status in the modern Food and Agriculture World (where most animal foods, except fish, are lacking ω-3), things are easy only apparently. It could be claimed that we should eat more fatty fish, more tree

nuts, and more rapeseed oil. In fact, this means that the list of ω-3 rich foods is very short. In addition, these foods, especially fish and tree nuts, are quite expensive and not available everywhere, many people do not like them or cannot eat them for various reasons. It is therefore important to propose "alternative" ω-3 rich foods to the consumers. "Alternative" means here that these foods are produced in accordance with natural and traditional processes and considerably differ from the typical industrial foods made principally to respond to economic criteria; a good example are ω-3 rich eggs. Contrary to fish, eggs are among the foods present in the fridge of every household and are quite cheap. Eggs are a good source of proteins and vitamins and are well accepted in most cultures and societies. They can be cooked in various ways and used in many recipes. Thus, ω-3 rich eggs are probably a very appropriate way to provide ω-3 fatty acids to the modern consumers.

For instance, Columbus Eggs contain 550 mg ALA and 110 mg very long chain ω-3 (present in fish oil), thus a whole range of biologically active ω-3 fatty acids in an ideal 5:1 ratio between plant-type (C18, ALA) and animal-type (very long chain), as expressed by the 1999 International Expert Consultation on the Essentiality of and Dietary Reference Intake (DRIs) for ω-6 and ω-3 Fatty Acids. In addition, Columbus Egg is produced by hens the fat depots of which is characterized by a 1:1 ratio between the two essential ω-6 (linoleic acid) and ω-3 (ALA) fatty acids, a characteristic of fat depots in game or wild animals. This ideal essential fatty acid composition of Columbus Egg makes it a very attractive food source of nutrients.

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17 Dietary Prevention of Coronary Heart Disease

Michel de Lorgeril and Patricia Salen

Abstract

Active prevention of coronary heart disease (CHD) should be started early in life and focus on prevention of arterial disease. Secondary prevention however should primarily focus on the risk of death and myocardial protection. The two main causes of death in these patients are sudden cardiac death (SCD) and heart failure (HF), often resulting from myocardial ischemia and subsequent necrosis. In that context, it is crucial to understand that our populations are chronically and severely deficient in some major nutrients in particular ω-3 polyunsaturated fatty acids (n-3 PUFA). Actually, consumption of n-3 PUFA is inversely correlated with the risk of SCD, the first cause of death in CHD patients. On the other hand, the main mechanism underlying recurrent cardiac events is myocardial ischemia resulting from atherosclerotic plaque rupture or ulceration. Plaque rupture is usually the consequence of intraplaque inflammation in relation with a high lipid content of the lesion, high concentration of leukocytes and lipid peroxidation products. Thus, in patients with established CHD, the three main aims of the preventive strategy are to prevent malignant ventricular arrhythmias and SCD *(1)*, the development of severe ventricular dysfunction and heart failure *(2)*, and to minimize the risk of plaque inflammation and ulceration *(3)*. For that purpose, the adoption of a Mediterranean diet rich in ω-3 fatty acids seems to be the most effective strategy.

Key Words: Coronary heart disease; heart failure; atherosclerosis; Mediterranean diet; ω-3 fatty acids; selenium.

1. DIETARY PREVENTION OF SUDDEN CARDIAC DEATH

Sudden cardiac death (SCD) is usually defined as death from a cardiac cause occurring within one hour from the onset of symptoms *(1)*. In many studies, however, investigators used quite different definitions with a time frame of 3 h or even 24 h in the old World Health Organization (WHO) definition. The magnitude of the problem is considerable as SCD is a very common, and often the first, manifestation of coronary heart disease (CHD), and it accounts for not less than 60% of cardiovascular mortality in developed countries *(1,2)*. In most cases, SCD occurs without prodromal symptoms and out of hospital. As a matter of fact, this mode of death is a major public health issue. Because as many as 80% of SCD patients had CHD *(1)*, the epidemiology and potential preventive approaches of SCD should, in theory, parallel those of CHD. In other words, any treatment aimed at reducing CHD should reduce the incidence of SCD.

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1.1. Fish, n-3 Fatty Acids, and SCD

The hypothesis that eating fish may protect against SCD is derived from the results of a secondary prevention trial, the Diet And Reinfarction Trial (DART), which showed a significant reduction in total and cardiovascular mortality (both by about 30%) in patients who had at least 2 servings of fatty fish/wk *(3)*. The authors suggested that the protective effect of fish might be explained by a preventive action on ventricular fibrillation (VF), because no benefit was observed on the incidence of nonfatal AMI. This hypothesis was consistent with experimental evidence suggesting that n-3 polyunsaturated fatty acids (PUFAs), the dominant fatty acids in fish oil and fatty fish, have an important effect on the occurrence of VF in the setting of myocardial ischemia and reperfusion in various animal models, both in vivo and in vitro *(4)*. In the same studies, it was also apparent that saturated fatty acids are proarrhythmic as compared with unsaturated fatty acids. Using an elegant in vivo model of SCD in dogs, Billman demonstrated a striking reduction of VF after intravenous (iv) administration of pure n-3 PUFA, including both the long chain fatty acids present in fish oil and α-linolenic acid, their parent n-3 PUFAs occurring in some vegetable oils *(4)*. These authors have found the mechanism of this protection to result from the electrophysiological effects of free n-3 PUFAs when these are simply partitioned into the phospholipids of the sarcolemma without covalently bonding to any constituents of the cell membrane. After dietary intake, these fatty acids are preferentially incorporated into membrane phospholipids. It has also been shown that a very important pool of free (nonesterified) fatty acids exists in the normal myocardium and that the amount of n-3 PUFAs in this pool is increased by supplementing the diet in n-3 PUFAs. This illustrates the potential of diet to modify the structure and biochemical composition of cardiac cells. In case of ischemia, phospholipases and lipases quickly release new fatty acids from phospholipids, including n-3 fatty acids in higher amounts than the other fatty acids, thus further increasing the pool of free n-3 fatty acids that can exert an antiarrhythmic effect. It is important to remember that the lipoprotein lipase is particularly active following the consumption of n-3 PUFAs. One hypothesis is that the presence of the free form of the n-3 PUFA in the membrane of every cardiac muscle cell renders the myocardium more resistant to arrhythmias, probably by modulating the conduction of several membrane ion channels. So far, it seems that the very potent inhibitory effects of n-3 PUFA on the fast sodium current, INa, and the L-type calcium current, ICaL *(4)*, are the major contributors to the antiarrhythmic actions of these fatty acids in ischemia. Briefly, n-3 PUFAs act by shifting the steady-state inactivation potential to more negative values, as was also observed in other excitable tissues such as neurons *(4)*.

Another major aspect of that question is that most Western populations are severely deficient in ω-3 fatty acids from both the marine and terrestrial world. In the United States (US), experts of the American Heart Association (AHA) claim that the average consumption of ω-3 fatty acids should be increased four times to attain the recommended intakes *(5)*. Intake of vegetable ω-3 should be doubled. The same trends are observed in European countries such as France. Chronic deficiency in ω-3 fatty acids probably explains at least partly the high prevalence of cardiac death (and SCD) in these countries.

Another important aspect of the implication of n-3 PUFAs in SCD is their role in the metabolization of eicosanoids. In competition with n-6 PUFAs, they are the precursors

to a broad array of structurally diverse and potent bioactive lipids (including eicosanoids, prostaglandins and thromboxanes), which are thought to play a role in the occurrence of VF during myocardial ischemia and reperfusion. These fatty acids (both n-6 PUFAs and n-3 PUFAs) also play a role in the development of vascular inflammation and atherosclerosis through the leucotriene pathway *(6)*.

Other clinical data show suppression (by more than 70%) of ventricular premature complexes in middle-aged patients with frequent ventricular extrasystoles randomly assigned to take either fish oil or placebo. Also, survivors of AMI and healthy men receiving fish oil were shown to improve their measurements of heart rate variability, suggesting other mechanisms by which n-3 PUFAs may be antiarrhythmic. Support for the hypothesis of a clinically significant antiarrhythmic effect of n-3 PUFAs in the secondary prevention of CHD, as put forward in DART *(3)*, came from two randomized trials testing the effect of ethnic dietary patterns instead of that a single food or nutrient, (i.e., a Mediterranean type of diet and an Asian vegetarian diet, in the secondary prevention of CHD) *(1,7)*. The two experimental diets included a high intake of essential alpha-linolenic acid, the main vegetable n-3 PUFAs. Whereas the incidence of SCD was markedly reduced in both trials, the number of cases was small and the antiarrhythmic effect cannot be entirely attributed to α-linolenic acid as these experimental diets were also high in other nutrients with potential antiarrhythmic properties, including various antioxidants. These findings were extended by the population-based case-control study conducted by Siscovick and colleagues on the intake of n-3 PUFAs among patients with primary cardiac arrest, compared with that of age- and sex-matched controls *(8)*. These data confirm the very low consumption of n-3 PUFAs in the Western populations (especially in comparison with the Japanese) and indicated that the intake of about 5 to 6 g of n-3 PUFAs/mo (an amount provided by consuming fatty fish once or twice/wk) was associated with a 50% reduction in the risk of cardiac arrest. In that study, the use of a biomarker, the red blood cell membrane level of n-3 PUFAs, considerably enhanced the validity of the findings, which also were consistent with the results of many (but not all) cohort studies suggesting that consumption of one to two servings of fish per week is associated with a marked reduction in CHD mortality as compared with no fish intake. In most studies, however, the SCD endpoint is not reported.

In a large prospective study (more than 20,000 participants with a follow-up of 11 yr), Albert et al. examined the specific point that fish has antiarrhythmic properties and may prevent SCD *(9)*. Again these investigators found a very low average consumption of n-3 PUFAs. They found that the risk of SCD was 50% lower for men who consumed fish at least once/wk than for those who had fish less than once/mo. Interestingly, the consumption of fish was not related to non-sudden cardiac death suggesting that the main protective effect of fish (or n-3 PUFAs) is related to an effect on arrhythmia.

The GISSI-Prevenzione trial was aimed at helping in addressing the question of the health benefits of foods rich in n-3 PUFAs (and also in vitamin E) and their pharmacological substitutes *(10)*. Patients (n = 11,324) surviving a recent AMI (<3 mo) and having received the prior advice to come back to a Mediterranean type of diet were randomly assigned supplements of n-3 PUFAs (0.8 g/d), vitamin E (300 mg/d), both, or none (control) for 3.5 yr. The primary efficacy endpoint was the combination of death and nonfatal AMI and stroke. Secondary analyses included overall mortality, cardiovascular (CV) mortality and SCD. Treatment with n-3 PUFAs significantly lowered the risk

of the primary endpoint (the relative risk decreased by 15%). Secondary analyses provided a clearer profile of the clinical effects of n-3 PUFAs. Overall mortality was reduced by 20% and CV mortality by 30%. However, it was the effect on SCD (45% lower) that accounted for most of the benefits seen in the primary combined endpoint and both overall and CV mortality. There was no difference across the treatment groups for nonfatal CV events, a result comparable with that of DART *(3)*. Thus, the results obtained in this randomized trial are consistent with previous controlled trials *(1)*, large-scale observational studies *(8,9)*, and experimental studies *(4)*, which together strongly support an effect of n-3 PUFAs in relation with SCD.

Regarding the physiopathology, administering n-3 PUFAs iv to patients with an ICD (implanted defibrillator) and at very high risk of SCD seems to provide those patients with an extraordinary degree of protection against malignant ventricular arrhythmias *(11)*. Recently, Leaf and colleagues reported that the main protective effect of very long chain n-3 PUFAs, EPA, and DHA probably results from the prevention of malignant arrhythmias. As a matter of fact, in the Fatty Acid Anti-Arrhythmia Trial (FAAT) conducted in more than 400 patients at high risk of SCD (and with ICDs), patients randomized to receive (double blind protocol) a mix of 2.4 g of EPA + DHA had a 40% reduction of the risk of malignant ventricular arrhythmias as recorded by the ICD compared with those receiving the placebo *(12)*.

1.2. Saturated Fatty Acids, Oleic Acid, Trans *Fatty Acids, and n-6 Fatty Acids*

Regarding the other dietary fatty acids, animal experiments have clearly indicated that a diet rich in saturated fatty acids is associated with a high incidence of ischemia- and reperfusion-induced ventricular arrhythmia, whereas PUFA of either the n-6 or n-3 family reduce that risk *(4)*. Large (but not all) epidemiological studies have shown consistent associations between the intake of saturated fatty acids and CHD mortality *(1)*. However, the SCD endpoint is usually not analysed in these studies. In addition, a clear demonstration of a causal relationship between dietary saturated fatty acids and SCD would require the organization of a randomized trial, which is not ethically acceptable. Thus, besides the effect of saturated fatty acids on blood cholesterol levels, the exact mechanism(s) by which saturated fats increase CHD mortality remain unclear. If animal data, demonstrating a proarrhythmic effect of saturated fatty acids, are confirmed in humans, the first thing to do in order to prevent SCD in humans would be to drastically reduce the intake of saturated fats. In fact, this has been done in randomized dietary trials and, as expected, the rate of SCD decreased in the experimental groups *(1)*. However, as written above about the same trials *(1)*, the beneficial effect cannot be entirely attributed to the reduction of saturated fats, because other potentially antiarrhythmic dietary factors, including n-3 PUFAs, were also modified in these trials.

In contrast with n-3 PUFAs, few data have been published so far in humans regarding the effect of n-6 PUFAs on the risk of SCD. Roberts et al. have reported that the percentage content of linoleic acid (the dominant n-6 PUFAs in the diet) in adipose tissue (an indicator of long term dietary intake) was inversely related to the risk of SCD, which was defined in that study as instantaneous death or death within 24 h of the onset of symptoms *(1)*. This is in line with most animal data and may suggest that patients at risk of

SCD may benefit from increasing their dietary intake of n-6 PUFAs, in particular linoleic acid, in the same way as for n-3 PUFAs. Let it be mentioned, however, that n-3 PUFAs were more effective on SCD than n-6 PUFAs in most animal experiments *(4)*.

In addition, diets high in n-6 PUFAs increase the linoleic acid content of lipoproteins and render them more susceptible to oxidation, which would be an argument against such diets because lipoprotein oxidation is a major step in the inflammatory process that renders atherosclerotic lesions unstable and prone to rupture.

Erosion and rupture of atherosclerotic lesions were shown to trigger CHD complications (*see* Section 3) and myocardial ischemia and to considerably enhance the risk of SCD. As a matter of fact, in the secondary prevention of CHD, diets high in n-6 PUFAs failed to improve the overall prognosis of the patients. Also, in the Dayton study (the Los Angeles Veteran trial), a mixed primary and secondary prevention trial, in which the chief characteristic of the experimental diet was the substitution of n-6 PUFAs for saturated fat, the number of SCD was apparently lower in the experimental group than in the control group (18 vs 27) but the number of deaths from other causes, in particular cancers, was higher in the experimental group (85 vs 71), thus offsetting the potential protective effect of n-6 PUFAs on SCD and resulting in no effect at all on mortality. Such negative effects were not reported with n-3 PUFAs. Thus, despite the beneficial effect of n-6 PUFAs on lipoprotein levels, which could, in theory, reduce SCD in the long term by reducing the development of atherosclerosis, it seems preferable not to increase the consumption of n-6 PUFA beyond the amounts required to prevent deficiencies in the essential n-6 fatty acid, linoleic acid (approx 4–6% of the total energy intake), which are found in the current average Western diet. As a substitute for saturated fat, the best choice is obviously to increase the intake of vegetable monounsaturated fat (e.g., oleic acid) in accordance with the Mediterranean diet pattern. If oleic acid has apparently no effect on the risk of SCD (at least by comparison with n-3 and n-6 PUFAs), its effects on blood lipoprotein levels are similar to those of n-6 PUFA and it has the great advantage of protecting lipoproteins against oxidation.

Thus, the best fatty acid combination to prevent SCD (and the other complications of CHD) and, in other words, to cumulate antiarrhythmic, antioxidant, and hypolipidemic effects, would result from the adoption of a diet close to the Mediterranean diet pattern.

Finally, Roberts et al. reported no significant relationship between *trans* isomers of oleic and linoleic acids in adipose tissue and the risk of SCD whereas Lemaitre et al. found that cell membrane *trans* isomers of linoleic acid (but not of oleic acid) are associated with a large increase in the risk of primary cardiac arrest. As for the role of *trans* fatty acids on ventricular arrhythmias, it has not been investigated in experimental models.

Thus, although specific human data on the effect of saturated fatty acids on SCD are lacking, results of several trials suggest that it is important to reduce their intake in the secondary prevention of CHD. Despite a possible beneficial effect on the risk of SCD, increasing consumption of n-6 PUFAs should not be recommended in clinical practice for patients with established CHD. Diets including low intakes in saturated fatty acid (as well as *trans* isomers of linoleic acid) and n-6 PUFAs (but enough to provide the essential linoleic acid) and high intakes in n-3 PUFAs and oleic acid (Mediterranean diet pattern) appear to be the best option to prevent both SCD and nonfatal AMI recurrence *(13)*.

1.3. Alcohol and SCD

The question of the effect of alcohol on heart and vessel diseases has been the subject of intense controversy in recent years. The consensus is now that moderate alcohol drinking is associated with reduced cardiovascular mortality, although the exact mechanism(s) by which alcohol is protective are still unclear. In experimental models, an ethanol preconditioning phenomenon *(14)* has been reported suggesting that low-dose ethanol drinking protects the myocardium against damages provoked by ischemia. In contrast, chronic heavy drinking has been incriminated in the occurrence of atrial as well as ventricular arrhythmias in humans, an effect called "the holiday heart" because it is often associated with binge drinking by healthy people, specifically during the weekend. Studies in animals have shown varying and apparently contradictory effects of alcohol on cardiac rhythm and conduction, depending on the animal species, the experimental model and the dose of alcohol. If given acutely to nonalcoholic animals, ethanol may even have antiarrhythmic properties.

In humans, few studies have specifically investigated the effect of alcohol on SCD. The hyperadrenergic state resulting from binge drinking, as well as from withdrawal in alcoholics, seems to be the main mechanism by which alcohol induces arrhythmias in humans. In the British Regional Heart Study, the relative risk of SCD in heavy drinkers (>6 drinks/d) was twice as high as in occasional or light drinkers. However, the effect of binge drinking on SCD was more evident in men with no pre-existing CHD than in those with established CHD. In contrast, in the Honolulu Heart Program, the risk of SCD among healthy middle-aged men was positively related to blood pressure, serum cholesterol, smoking, and left ventricular hypertrophy but inversely related to alcohol intake. In fact, the effect of moderate "social" drinking on the risk of SCD in nonalcoholic subjects has been addressed so far in only one study. Investigators of the Physicians' Health Study assessed whether light-to-moderate alcohol drinkers apparently free of CHD at baseline have a decreased risk of SCD *(15)*. After controlling for multiple confounders, men who consumed between 2 and 4 drinks/wk or 5 and 6 drinks/wk at baseline had a significantly reduced risk of SCD (by 60 to 80%) as compared with those who rarely or never consumed alcohol. Analyses were repeated after excluding deaths occurring during the first 4 yr of follow-up (in order to exclude the possibility that some men who refrained from drinking at baseline did so because of early symptoms of heart diseases), and also using the updated measure of alcohol intake ascertained at year 7 to address potential misclassification in the baseline evaluation of alcohol drinking *(15)*. These secondary analyses basically provided the same results and confirmed the potential protective effect of moderate drinking on the risk of SCD. Despite limitations (the selected nature of the cohort, an exclusively male study group, no information on beverage type and drinking pattern), this study suggests that a significant part of the cardioprotective effect of moderate drinking is related to the prevention of SCD. Further research should be directed at understanding the mechanism(s) by which moderate alcohol drinking may prevent ventricular arrhythmias and SCD.

In practice, current state knowledge suggests that in CHD patients at risk of SCD, there is no reason not to allow moderate alcohol drinking. From a practical point of view, we advise to drink one or two drinks/d, preferably wine, preferably during the evening meal, and never before driving a car or making a dangerous work.

1.4. Cholesterol Lowering and SCD

Another important issue at a time when so many people are taking cholesterol-lowering drugs with the hope to improve their life expectancy is whether cholesterol lowering might reduce the risk of SCD. According to recent data standardized to the 2000 US population *(2)*, of 719,456 cardiac deaths among adults aged >35 yr, 63% were defined as SCD. In that study, SCD was defined as death occurring out of the hospital or in the emergency room or as "dead on arrival" with an underlying cause of death reported as a cardiac disease. Among those aged 35 to 55, about 75% of cardiac deaths were SCD *(2)*.

Another question is: are we able to identify people at risk of SCD? In other words, are the traditional risk factors of CHD predictive of SCD? Several studies have recently tried to answer that question. For instance, in a prospective study in healthy men, investigators found that only C-reactive protein (CRP) was significantly associated with the risk of SCD whereas homocysteine and all lipid parameters, including total and LDL cholesterol levels, were not *(16)*. In another study investigating the determinants of SCD in women, diabetes and smoking conferred markedly elevated risk of SCD whereas hypercholesterolemia did not increase the risk of SCD *(17)*. Thus, it seems that high cholesterol level is not a risk factor of SCD whereas SCD appears to be the main cause of CHD death.

If these epidemiological data are true, the next obvious question is whether (or in which proportions) cholesterol lowering is able to reduce the risk of SCD and consequently the risk of CHD death. As the best cholesterol lowering treatments are the statin drugs, it is important to look at the SCD data (and CHD mortality data) in the recently published statin trials. However, in most (recent as well as old) statin trials, there is curiously no data regarding the effect of statins on SCD. This suggests that, as expected from epidemiological data, statins had no significant effect on SCD. Given the importance of SCD as a cause of death in CHD patients, we can therefore suspect that the effect of cholesterol lowering by statins on CHD death was, at best, small. In fact, when published trials are carefully reviewed, it appears that the effect of statins on mortality was either small or nonsignificant. For instance, in HPS, PROSPER, ALLHAT-LLT, ASCOT-LLA, and ALLIANCE, the death rate ratios were 0.87 (indicating a risk reduction of 13%), 0.97 (nonsignificant), 0.99 (nonsignificant), 0.87 (nonsignificant), and 0.92 (non significant) respectively *(18–22)*. Furthermore, the effect of statins specifically in women has been recently analyzed using a meta-analysis of 13 studies retained in the Cochrane database *(23)*. The authors concluded that in both primary and secondary prevention, statins had no significant effect on mortality in women. Finally, in the most recent statin trials focusing on patients with acute CHD syndromes and early and intensive lipid lowering (MIRACL, PROVE-IT, A to Z Trial), the effects on mortality were again small or nonsignificant despite the recruitment of several thousand patients in these trials *(24,25)*. Thus, cholesterol lowering with statins does not appear to be a very effective way of reducing CHD mortality in our populations. This is not unexpected as the main cause of CHD death is SCD (up to 75% of cardiac death among people aged 35–55) and SCD is apparently not determined by lipid factors.

2. DIET AND THE RISK OF HEART FAILURE FOLLOWING AMI

The incidence of chronic heart failure (CHF), the common end-result of most cardiac diseases, is increasing steadily in many countries despite (and probably because of)

considerable improvements in the acute and chronic treatment of CHD, which is nowadays the main cause of CHF in most countries *(26)*. In the recent years, most research effort about CHF has been focused on drug treatment, and there has been little attention paid to nonpharmacological management. Some unidentified factors may indeed contribute to the rise in the prevalence of CHF and should be recognized and corrected if possible. For instance, CHF is now seen also as a metabolic problem with endocrine and immunological disturbances potentially contributing to the progression of the disease. In particular, the role of the tumor necrosis factor (TNF) is discussed below. Only recently has it been also recognized that increased oxidative stress may contribute to the pathogenesis of CHF. The intimate link between diet and oxidative stress is obvious, knowing that the major antioxidant defences of our body are derived from essential nutrients.

Whereas it is generally considered that a high sodium diet is detrimental (and may result in acute decompensation of heart failure through a volume overload mechanism), little is known about other aspects of diet in CHF in terms of both general nutrition and micronutrients such as vitamins and minerals. In these patients, it is important not only to take care of the diagnosis and treatment of the CHF syndrome itself and for the identification and aggressive management of traditional risk factors of CHD such as high blood pressure and diabetes (because they can aggravate the syndrome), but also for the recognition and correction of malnutrition and of deficiencies in specific micronutrients.

The vital importance of micronutrients for health and the fact that several micronutrients have antioxidant properties are now fully recognized. These may be as direct antioxidants such as vitamins C and E or as components of antioxidant enzymes: superoxide dismutase or glutathione peroxidase. It is now widely believed (but still not causally demonstrated) that diet derived antioxidants may play a role in the development (and thus in the prevention) of CHF. For instance, clinical and experimental studies have suggested that CHF may be associated with increased free radical formation and reduced antioxidant defences and that vitamin C may improve endothelial function in patients with CHF. In the secondary prevention of CHD, in dietary trials in which the tested diet included high intakes of natural antioxidants, the incidence of new episodes of CHF was reduced in the experimental groups. Taken altogether, these data suggest (but do not demonstrate) that antioxidant nutrients may help prevent CHF in postinfarction patients.

Other nutrients, however, may be also involved in certain cases of CHF. Whereas deficiency in certain micronutrients, whatever the reason, can actually cause CHF and should be corrected, it is important to understand that patients suffering from CHF also have symptoms that can affect their food intake and result in deficiencies, for instance tiredness when strained, breathing difficulties, and gastrointestinal symptoms such as nausea, loss of appetite and early feeling of satiety. Drug therapy can lead to loss of appetite and excess urinary losses in case of diuretic use. All of these are mainly consequences, not causative factors, of CHF. Thus the basic treatment of CHF should, in theory, improve these nutritional anomalies. However, since they can contribute to the development and severity of CHF, they should be recognized and corrected as early as possible.

Finally, it has been shown that up to 50% of patients suffering from CHF are malnourished to some degree, and CHF is often associated with weight loss. There may be multiple etiologies to the weight loss, in particular lack of activity resulting in loss of

muscle bulk and increased resting metabolic rate. There is also a shift towards catabolism with insulin resistance and increased catabolic relative to anabolic steroids. TNF, sometimes called cachectin, is higher in many patients with CHF, which may explain weight loss in these patients. Interestingly, there is a positive correlation between TNF and markers of oxidative stress in the failing heart suggesting a link between TNF and antioxidant defences in CHF (the potential importance of TNF in CHF is discussed below in the section on dietary fatty acids and CHF). Finally, cardiac cachexia is a well recognized complication of CHF, its prevalence increases as symptoms worsen and it is an independent predictor of mortality in CHF patients. However, the pathophysiological alteration leading to cachexia remains unclear and at present, there is no specific treatment apart from the treatment of the basic illness and correction of the associated biological abnormalities.

2.1. Deficiency in Specific Micronutrients

As written above, an important practical point is that deficiencies in specific micronutrients can actually cause CHF, or at least aggravate it. The prevalence of these deficiencies among patients with CHF (and postinfarction patients) is unknown. Whether we should systematically search for them also remains unclear. In particular, we do not know whether the association of several borderline deficiencies that do not individually result in CHF may result in CHF, especially in the elderly. For certain authors, however, there is sufficient evidence to support a large-scale trial of dietary micronutrient supplementation in CHF.

There is no room here to fully expose the present knowledge in that field. Nonetheless, if we restrict our comments only to human data, things can be summarized as follows. Cases of hypocalcemia-induced cardiomyopathy (usually in children with a congenital cause for hypocalcemia) that can respond dramatically to calcium supplementation have been reported.

Hypomagnesemia is often associated with a poor prognosis in CHF, and correction of the magnesium levels (in anorexia nervosa for instance) leads to an improvement in cardiac function. Low serum and high urinary zinc levels are found in CHF, possibly as a result of diuretic use, but there are no data regarding the clinical effect of zinc supplementation in that context. In a recent study, plasma copper was slightly higher and zinc slightly lower in CHF subjects than in healthy controls. As expected, dietary intakes were in the normal range and no significant relationship was found between dietary intakes and blood levels in the 2 groups. It is not possible to say whether these copper and zinc abnormalities may contribute to the development of CHF or are simple markers for the chronic inflammation known to be associated with CHF. Further studies are needed to address the point, since the implications for prevention are substantial.

Selenium deficiency has been identified as a major factor in the etiology of certain nonischemic CHF syndromes, especially in low-selenium soil areas such as Eastern China and Western Africa. In Western countries, cases of congestive cardiomyopathy associated with low-antioxidant nutrients (vitamins and trace elements) have been reported in malnourished HIV infected patients and in subjects on chronic parenteral nutrition. Selenium deficiency is also a risk factor for peripartum cardiomyopathy. In China, an endemic cardiomyopathy called Keshan disease seems to be a direct consequence of selenium deficiency. Whereas the question of the mechanism by which selenium deficiency results

in CHF remains open, recent data suggest that selenium may be involved in skeletal (and cardiac) muscle deconditioning (and in CHF symptoms such as fatigue and low exercise tolerance) rather than in left ventricular dysfunction. Actually, in the Keshan area, the selenium status coincides with the clinical severity rather than with the degree of left ventricular dysfunction as assessed by echocardiographic studies. When the selenium levels of residents were raised to the typical levels in the nonendemic areas, the mortality rate declined significantly but clinically latent cases were still found and the echocardiographic prevalence of the disease remained high. What we learned from Keshan disease, and other studies conducted elsewhere, is that in patients with a known cause of CHF even a mild deficiency in selenium may influence the clinical severity of the disease (tolerance to exercise).

These data should serve as a strong incentive for the initiation of studies testing the effects of natural antioxidants on the clinical severity of CHF. In the meantime, however, physicians would be well advised to measure selenium in patients with an exercise inability disproportionate to their cardiac dysfunction.

Finally, low whole-blood thiamine (vitamin B1) levels have been documented in patients with CHF on loop diuretics and hospitalized elderly patients, and thiamine supplementation induced a significant improvement in cardiac function and symptoms.

2.2. Dietary Fatty Acids and Sodium Intake, Cytokines, LVH, and CHF

Beyond the well known effect of high sodium intake in the clinical course of CHF (and the occurrence of acute episodes of decompensation), another important issue is the role of diet in the development of left ventricular hypertrophy (LVH), a major risk factor for CHF (and also SCD), as well as for cardiovascular and all-cause mortality and morbidity.

The cause of LVH is largely unknown. Whereas male gender, obesity, heredity, and insulin resistance may explain some of the variance in LVH, hypertension (HBP) is generally regarded as the primary culprit. Thus, the risks associated with LVH and HBP are intimately linked. Recent data did also suggest that low dietary intake of PUFAs and high intake of saturated fatty acids, as well as HBP and obesity, at age 50 predicted the prevalence of LVH 20 yr later. Although the source of saturated fatty acids is usually animal fat, the source of unsaturated fatty acids in that specific Scandinavian population and at that time was less clear and there was no adjustment for other potential dietary confounders such as magnesium, potassium, calcium, and sodium. Thus, this study did not provide conclusive data to deinitively conclude the dietary lipid determinants of LVH. However, it did suggest that dietary fatty acids may be involved in the development of LVH and that this "diet-heart connection" may partly explain the harmful effect of animal saturated fatty acids on the heart.

Another "diet-heart connection" in the context of advanced CHF relates to the recent theory that CHF also is a low-grade chronic inflammatory disease with elevated circulating levels of cytokines and cytokine receptors that are otherwise independent predictors of mortality. High-dose ACE-inhibition with enalapril, a treatment that reduces mechanical overload and shear stress (two stimuli for cytokine production in patients with CHF), was recently shown to decrease both cytokine bioactivity and left ventricular wall thickness. Finally, various anticytokine and immunomodulating agents were shown to have beneficial effect on heart function and clinical functional class in

patients with advanced CHF suggesting a causal relationship between high cytokine production and CHF. This also suggests that there is a potential for therapies altering cytokine production in CHF. In that regard, it has been shown that dietary supplementation with n-3 fatty acids (either fish oil or vegetable oil rich in n-3 fatty acid) reduces cytokine production at least in healthy volunteers. An inverse exponential relationship between leukocyte n-3 fatty acid content and cytokine production by these cells was found, most of the reduction in cytokine production being seen with eicosapentanoic acid in cell membrane lower than 1%, a level obtained with rather moderate n-3 fatty acid supplementation. However, further studies are warranted to test whether (and at which dosage) dietary n-3 fatty acids may influence the clinical course of CHF through an anticytokine effect.

Sodium intake is the environmental factor that is currently most suspected of influencing blood pressure and the prevalence of high blood pressure. However, the full damaging potential of high sodium intake for the heart (and also the kidney) seems to be largely independent of the pressor effect of sodium. Animal experiments and clinical studies have consistently shown that high sodium intake is a powerful and independent determinant of LVH and that such an arterial-pressure-independent effect of salt is not confined to the heart.

Whereas the long-term effect of a reduced sodium intake after a recent AMI is unknown, in particular on LVH, experts claim that even a 50 mmol reduction in the daily sodium intake would reduce the average systolic blood pressure by at least 5 mmHg (in patients aged over 50 yr) and CHD mortality by about 16%. Thus, as regards the damaging effect of high sodium intake on the heart, and despite the lack of strong data showing the beneficial effect of reducing sodium intake in that specific group of patients, we believe that cardiologists should extend their dietary counselling about sodium not only to the patients with HBP or CHF but also to all post-infarction patients.

3. DIET AND THE PREVENTION OF PLAQUE INFLAMMATION AND RUPTURE

For several decades, the prevention of CHD (including the prevention of ischemic recurrence after a prior AMI) has focused on the reduction of the traditional risk factors: smoking, high blood pressure, and hypercholesterolemia. Priority was given to the prevention (or reversion) of vascular atherosclerotic stenosis. As discussed above, it has become clear in secondary prevention that clinical efficiency needs to primarily prevent the fatal complications of CHD such as SCD. This does not mean, however, that we should not try slowing down the atherosclerotic process, and in particular plaque inflammation and rupture. Indeed, it is critical to prevent the occurrence of new episodes of myocardial ischemia whose repetition in a recently injured heart can precipitate SCD or CHF. Myocardial ischemia is usually the consequence of coronary occlusion caused by plaque rupture and subsequent thrombotic obstruction of the artery. Recent progress in the understanding of the cellular and biochemical pathogenesis of atherosclerosis suggests that, in addition of the traditional risk factors of CHD, there are other very important targets of therapy to prevent plaque inflammation and rupture. In this regard, the most important question is: how and why does plaque rupture occur?

3.1. CHD is an Inflammatory Disease

Most investigators agree that atherosclerosis is a chronic low-grade inflammation disease *(27)*. Proinflammatory factors (e.g., free radicals produced by cigarette smoking, hyperhomocysteinemia, diabetes, peroxidized lipids, hypertension, elevated, and modified blood lipids) contribute to injure the vascular endothelium, which results in alterations of its antiatherosclerotic and antithrombotic properties. This is thought to be a major step in the initiation and formation of arterial fibrostenotic lesions. From a clinical point of view, however, an essential distinction should be made between unstable, lipid-rich and leukocyte-rich lesions and stable, acellular fibrotic lesions poor in lipids, as the propensity of these two types of lesion to rupture into the lumen of the artery, whatever the degree of stenosis and lumen obstruction, is totally different.

In 1987 we proposed that inflammation and leukocytes play a role in the onset of acute CHD events *(28)*. This has recently been confirmed. It is now accepted that one of the main mechanisms underlying the sudden onset of acute CHD syndromes—including unstable angina, myocardial infarction, and SCD—is the erosion or rupture of an atherosclerotic lesion, which triggers thrombotic complications and considerably enhances the risk of malignant ventricular arrhythmias. Leukocytes have been also implicated in the occurrence of ventricular arrhythmias in clinical and experimental settings, and they contribute to myocardial damage during both ischemia and reperfusion. Clinical and pathological studies showed the importance of inflammatory cells and immune mediators in the occurrence of acute CHD events and prospective epidemiological studies showed a strong and consistent association between acute CHD and systemic inflammation markers. A major question is to know why there are macrophages and activated lymphocytes in atherosclerotic lesions and how they get there. Issues such as local inflammation, plaque rupture, and attendant acute CHD complications follow.

3.2. The Lipid Oxidation Theory of CHD

Steinberg et al. proposed in 1989 that oxidation of lipoproteins causes accelerated atherogenesis *(29)*. Elevated plasma levels of low-density lipoproteins (LDL) are a major factor of CHD, and reduction of blood LDL levels (for instance by drugs) results in less CHD. However, the mechanism(s) behind the effect of high LDL levels is not fully understood. The concept that LDL oxidation is a key characteristic of unstable lesions is supported by many reports. Two processes have been proposed. First, when LDL particles become trapped in the artery wall, they undergo progressive oxidation and are internalized by macrophages, leading to the formation of typical atherosclerotic foam cells. Oxidized LDL is chemotactic for other immune and inflammatory cells and upregulates the expression of monocyte and endothelial cell genes involved in the inflammatory reaction. The inflammatory response itself can have a profound effect on LDL, creating a vicious circle of LDL oxidation, inflammation, and further LDL oxidation. Second, oxidized LDL circulates in the plasma for a long enough period to enter and accumulate in the arterial intima, suggesting that the entry of oxidized lipoproteins within the intima may be another mechanism of lesion inflammation, in particular in patients without hyperlipidemia. Elevated plasma levels of oxidized LDL are associated with CHD, and the plasma level of malondialdehyde-modified LDL is higher in patients with unstable CHD syndromes (usually associated with plaque rupture) than in patients with clinically stable CHD. In the accelerated form of CHD typical of post-transplantation patients,

higher levels of lipid peroxidation and of oxidized LDL were found as compared with the stable form of CHD in non-transplanted patients. Reactive oxygen metabolites and oxidants influence thrombus formation, and platelet reactivity is significantly higher in transplanted patients than in non-transplanted CHD patients.

The oxidized LDL theory is not inconsistent with the well established lipid-lowering treatment of CHD, as there is a positive correlation between plasma levels of LDL and markers of lipid peroxidation and low absolute LDL level results in reduced amounts of LDL available for oxidative modification. LDL levels can be lowered by drugs or by reducing saturated fats in the diet. Reduction of the oxidative susceptibility of LDL was reported when replacing dietary fat with carbohydrates. Pharmacological/quantitative (lowering of cholesterol) and nutritional/qualitative (high antioxidant intake) approaches of the prevention of CHD are not mutually exclusive but additive and complementary. An alternative way to reduce LDL concentrations is to replace saturated fats with polyunsaturated fats in the diet. However, diets high in PUFAs increase the PUFA content of LDL particles and render them more susceptible to oxidation (which would argue against use of such diets (*see* Section 1.2). As a matter of fact, in the secondary prevention of CHD, such diets failed to improve the prognosis of the patients. In that context, the traditional Mediterranean diet, with low saturated fat and polyunsaturated fat intakes, appears to be the best option. Diets rich in oleic acid increase the resistance of LDL to oxidation independent of the content in antioxidants and results in leukocyte inhibition. Thus, oleic acid-rich diets decrease the pro-inflammatory properties of oxidized LDL. Constituents of olive oil other than oleic acid may also inhibit LDL oxidation. Various components of the Mediterranean diet may also affect LDL oxidation. For instance, α-tocopherol or vitamin C, or a diet combining reduced fat, low-fat dairy products, and a high intake of fruits and vegetables were shown to favourably affect either LDL oxidation itself or/and the cellular consequences of LDL oxidation.

Finally, significant correlation was found between certain dietary fatty acids and the fatty acid composition of human atherosclerotic plaques, which suggests that dietary fatty acids are rapidly incorporated into the plaques. This implies a direct influence of dietary fatty acids on plaque formation and the process of plaque rupture. It is conceivable that fatty acids that stimulate oxidation of LDL (n-6 PUFAs) induce plaque rupture whereas those that inhibit LDL oxidation (oleic acid), inhibit leukocyte function (n-3 PUFAs) or prevent "endothelial activation" and the expression of proinflammatory proteins (oleic acid and n-3 fatty acids) contribute to pacify and stabilize the dangerous lesions. In that regard, it is noteworthy that moderate alcohol consumption, a well known cardioprotective factor, was recently shown to be associated with low blood levels of systemic markers of inflammation, suggesting a new protective mechanism to explain the inverse relationship between alcohol and CHD rate. In the same line, the potential of dietary n-3 fatty acids to reduce the production of inflammatory cytokines by leukocytes (as discussed in the section on dietary fatty acids and CHF) should be underlined. As both dietary n-3 fatty acids and moderate alcohol consumption are major characteristics of the Mediterranean diet, it is not surprising to observe that this diet was associated with lower rate of new episodes of CHF in the Lyon Diet Heart Study *(12)*.

Thus, any dietary pattern combining a high intake of natural antioxidants, a low intake of saturated fatty acids, a high intake of oleic acid, a low intake of ω-6 fatty acids, and a high intake of ω-3 fatty acids would logically produce a highly cardioprotective

effect. This is consistent with what we know about the Mediterranean diet pattern *(30)* and with the results of the Lyon Diet Heart Study and was recently confirmed by the GISSI investigators.

4. A MINIMUM CLINICAL PRIORITY DIETARY PROGRAM

Despite the increased evidence that dietary prevention is critical in the post-AMI patient, many physicians (and their patients) remain rather poorly informed about the potential of diet to reduce cardiac mortality, the risk of new CHD complications and the need for recurrent hospitalisation and investigation. There are many reasons for that, the main one probably being an insufficient knowledge of nutrition. For that reason (and knowing the resistance of many physicians to accept the idea that diet is important in CHD), we propose in the following lines *a minimum dietary program* that every CHD patient, whatever his/her medical and familial environment, should know and follow. This minimum "Mediterranean" should include the following:

1. Reduced consumption of animal saturated fat (i.e., by totally excluding butter and cream from the daily diet and drastic reduction of fatty meat) and increased consumption of n-3 fatty acids through increased intakes of fatty fish (a minimum of about 200 g, twice/wk). For patients who cannot eat fish (for any reason), taking capsules of n-3 fatty acids (for instance, a mix of α-linolenic acid and long-chain n-3 fatty acids) is the best alternative option. It is very important that the patients (and their physicians) be aware that n-3 fatty acid supplementation will be even more cardioprotective if associated with adequate dietary modifications discussed in the text above.
2. Increased intake of anti-inflammatory fatty acids (oleic acid and n-3 fatty acids) and decreased intake of proinflammatory fatty acids (n-6 fatty acids). The best way is to exclusively use olive oil and canola oil for cooking and salad dressing and canola oil-based margarine instead of butter and polyunsaturated oils and margarines. Patients should also systematically reject convenience food prepared with fats rich in saturated, polyunsaturated, and *trans* fatty acids.
3. Increased intake of natural antioxidants (vitamins and trace-elements) and folates through increased consumption of fresh fruits and vegetables and tree nuts.
4. Moderate intake of alcoholic beverages (1 or 2 drinks/d), preferably wine, preferably during the evening meal, and never before driving or making a dangerous technical manipulation.
5. Reduction of sodium intake (below 100 mmol/d if possible) knowing that it is a very difficult task at the present time because of the high sodium content of many natural (including typical Mediterranean foods such as olives and cheeses) and convenience food.

However, patients (and physicians) should keep in mind that optimal (and individual) dietary prevention program should be managed under the guidance of a professional dietician aware of the most recent scientific advances in the field.

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18 Fatty Acids, Insulin Resistance and Diabetes

Vijaya Juturu and James J. Gormley

Abstract

Recent data strongly suggest that atherosclerosis progression may be slowed by increased intakes of essential fatty acids (EFAs) from fish even in diabetes. The current evidence also indicates the potential benefits of EFAs on atrial fibrillation, particularly in the immediate post-cardiac surgery setting. EFAs also appear to reduce risk for arrhythmias and fatal coronary heart disease (CHD) events. Despite a growing body of research on the benefit of essential fatty acids (monounsaturated fatty acids [MUFAs] and polyunsaturated fatty acids [PUFAs]) for cholesterol lowering in CHD, there are insufficient outcome data on the use of EFAs on glycemic control as well as inadequate data to show the efficacy on insulin resistance and diabetes. The focus of this chapter is to discuss different fatty acids and therapeutic approaches toward treating insulin resistance syndrome (IRS) and diabetes exclusively by lowering cholesterol levels and glucose control as a medical nutrition therapy. Although results of epidemiologic studies as well as clinical trials and clinical end points confirm the association of (omega-3) fatty acids and MUFAs with cholesterol lowering, lowering inflammatory markers and risk of CHD, data are lacking regarding the effects of these fatty acids in the management of diabetes with CHD and insulin resistance. Type 2 diabetes mellitus (T2DM) is found to be associated with a two- to fourfold increase in CHD risk and as the degree of association of hyperglycemia is more related to microvascular complications, correcting dyslipidemia appears to reduce macro vascular events in patients with T2DM. Future dietary management strategies are required for the use of essential fatty acids in the management of insulin resistance and diabetes.

Key Words: Fatty acids; insulin resistance; diabetes; cardiovascular disease.

1. INTRODUCTION

Recent estimates suggest that insulin resistance syndrome (IRS) affects one in three people in the United States (US) (or 99.3 million Americans); in addition, 90% of individuals with diabetes are insulin resistant *(1)*. It has also been estimated that insulin resistance (IR)—which is a generalized metabolic disorder characterized by inefficient insulin function in skeletal muscle, liver and adipocytes—may occur in up to 25% of people without diabetes *(2)*. It is known that insulin resistance is associated with hypertension and cardiovascular disease (CVD) and that IR alone is responsible for 46.8, 6.2, and 12.5% of the annual coronary heart disease (CHD) events in type 2 diabetics, non-type 2 diabetics, and in the total US population, respectively.

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In the US, the annual total cost of events attributable to IR was estimated to be $12.5 billion in 1999, of which $6.6 billion were direct medical costs *(3)*. In addition, the annual total cost of diabetes jumped from $98 billion in 1997 to $132 billion in 2002.

IR is defined as an impaired ability of plasma insulin at usual concentrations to adequately promote peripheral glucose disposal, suppress hepatic glucose, and inhibit very low-density lipoprotein (VLDL) output. The existence of IR can be inferred based on strong clinical evidence confirmed by insulin and glucose measurements, including fasting insulin/glucose screening, oral glucose tolerance tests (OGTT), the minimal model frequently sampled intravenous (iv) glucose tolerance test (FSIVGTT), and insulin/glucose clamp studies. Fasting levels of insulin greater than 15 μU/mL, or insulin peak (post-OGTT) levels above 150 μU/mL and/or over 75 μU/mL at 120 min of OGTT are hyperinsulinemic levels, which indicate IR *(4)*. Insulin resistance may be associated with a cluster of modifiable risk factors such as blood pressure *(5)*, body weight and waist circumference *(6)*, dyslipidemia *(7,8)*, and impaired glucose tolerance *(9)*.

The two hormones produced by the pancreas—insulin and glucagon—maintain normal blood glucose. All hormones, including insulin and glucagon, exert their control over cells by stimulating production of hormone-like lipid compounds that serve local regulatory roles, called prostaglandins. In turn, prostaglandins communicate hormonal messages from hormones to individual cells. The prostaglandins are made from essential fatty acids (EFAs) and come in three series: series 1 (noninflammatory) and series 2 (inflammatory) from the linoleic (ω-6) fat group, and series 3 (noninflammatory) from the α-linolenic (ω-3) fat group. In addition to the fact that the modern Western diet is overbalanced in refined, proinflammatory ω-6 fats to the detriment of ω-3 consumption *(10)*, it is also typically high in saturated fats and trans fatty acids, fats that have been shown to decrease membrane fluidity and decrease insulin receptor binding, thus promoting insulin resistance *(11)*. In fact, the degree of insulin resistance correlates with the type of fatty acids that compose the cell membranes. Interestingly, Jeppson *(12)* reported that a diet with a high ω-6:ω-3 EFA ratio was detrimental to insulin receptor sensitivity. The high intake of soy oil, which is a predominantly ω-6 EFA, contributes to a ω-6:ω-3 fat imbalance in the US of up to 25:1, which, along with excess consumption of partially hydrogenated and saturated fats, is linked to the high prevalence of CVD, hypertension, type 2 diabetes mellitus (T2DM) and obesity, as well as other diseases.

It is becoming increasingly evident that specific fatty acids, or their derivatives, may have roles other than as energy substrates, and may also be involved in regulation of enzyme activity and gene expression in insulin-responsive tissues. Therefore, an increase in saturated fatty acids (SFA) and/or trans fatty acids (TFA) and a low status of EFAs (monounsaturated fatty acids [MUFAs] and poly unsaturated fatty acids [PUFAs]) may interfere with prostaglandin production. The EFA imbalance and proinflammatory state can intensify adult-onset diabetes regardless of whether adequate insulin is produced. There are indications that a high intake of fat is associated with impaired insulin sensitivity *(13–15)* and an increased risk of developing diabetes *(16–18)*.

Insulin stimulates lipogenesis in arterial tissue and adipose tissue via increased production of acetyl-coenzyme A (acetyl-CoA), increasing of triglycerides, and glucose. The characteristic dyslipidemia of raised triglycerides and reduced high-density lipoprotein (HDL) cholesterol apparently results from insulin's influence on the cholesteryl ester

transfer protein, which promotes the transfer of cholesteryl ester from HDL to very low-density lipoprotein (VLDL) cholesterol, which results in catabolism of apolipoprotein A-1 (Apo-A), the protein component of HDL. Insulin has also been shown to raise the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterologenesis, and thus may contribute to the raised cholesterol levels frequently seen with hyperinsulinemia *(19)*. The interconnection of insulin resistance to the development of conditions such as inflammation, endothelial dysfunction and atherosclerosis will be discussed.

Free fatty acids (FFAs) can cause hepatic insulin resistance *(20)*. It is known that FFAs support between 30 and 50% of basal insulin secretion and potentiate glucose-stimulated insulin secretion. The insulin-stimulatory action of FFAs is responsible for the fact that the vast majority (~80%) of insulin-resistant people who are obese do not develop type 2 diabetes. They are able to compensate for FFA-mediated insulin resistance with increased FFA-mediated insulin secretion. FFAs have recently been shown to activate the IκB/NFκB pathway, which is involved in many inflammatory processes. Thus, elevated plasma levels of FFAs are not only a major cause of insulin resistance in skeletal muscle and in the liver but may also play a role in the pathogenesis of coronary artery disease. Type 2 diabetes is characterized by hyperglycemia in the presence of insulin resistance, hypertriglyceridemia, and the development of vascular complications. Men and women with T2DM have three- and fivefold higher cardiovascular mortality, respectively, than the nondiabetic population, and nondiabetic first-degree relatives of T2DM subjects also carry this higher risk. Clustering of atherogenic (e.g., dyslipidemia, hypertension) and thrombotic (e.g., plasminogen activator inhibitor 1, factor VII, and fibrinogen) risk factors in association with insulin resistance may explain the higher risk.

Amri et al. *(21)* observed that fatty acids can regulate gene expression in adipose cells, introducing a link between the composition of diets and the hyperplastic and hypertrophic responses of white adipose tissue. The characterization of fatty acid–responsive genes may also produce some clues as to the pathogenesis of an insulin-resistant state and cell hypertrophy. This review focuses on the biological effects of different fatty acids on insulin resistance (*see* Fig. 1) and diabetes with the backdrop of evolutionary aspects of fatty acids in the diet.

2. EPIDEMIOLOGICAL DATA ON DIETARY FAT INTAKE

Borkman et al. *(22)* showed that hyperinsulinemia and insulin resistance are inversely associated with the amount of 20- and 22-carbon fatty acids in muscle cell membrane phospholipids in patients with CHD and in normal volunteers. In 2002, Hu et al. *(23)* reported results from the Nurses' Health Study, a prospective cohort study of female registered nurses (n = 84,688) with a 16-yr follow-up. The authors observed an inverse correlation between fish/ω-3 fatty acid consumption and incidence of CHD, including CHD death and nonfatal MI. In a subgroup analysis of diabetic nurses from this cohort *(24)*, a reduced risk of CHD from fish consumption was observed, although the association did not extend to estimated eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ω-3 fatty acid consumption. In a case-control study nested in the US Physicians Health Study *(25,26)* with a 17-yr follow-up, a significant inverse relationship between whole blood omega-3 fatty acid concentrations and CHD death was reported.

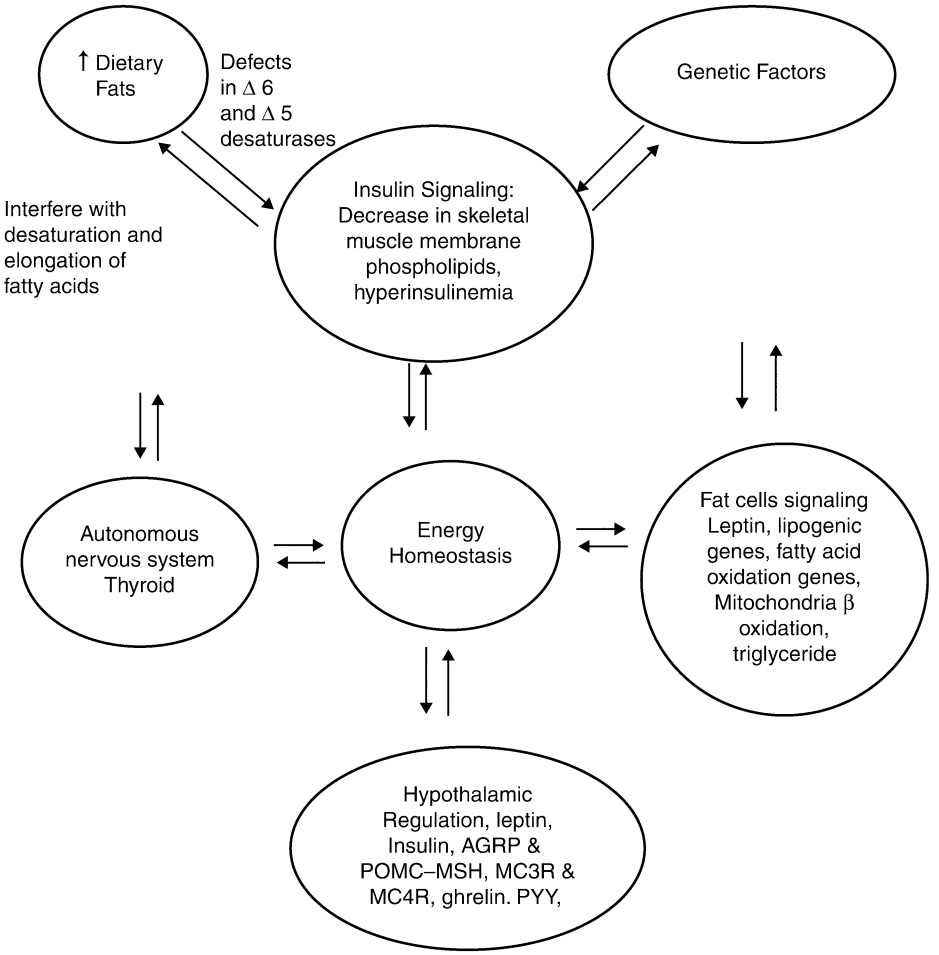

Fig. 1. Dietary fats enhances insulin resistance.

The prospective Kuopio Ischemic Heart Disease Risk Factor study *(27)*, which is part of World Health Organization's (WHO's) MONICA project, enrolled 1871 men who had no clinical CHD and observed a decrease in acute coronary events in men at the highest quintile of serum DHA + DPA concentration compared with men at the lowest quintile. In a prospective cohort study *(28)*, the estimated intake of EPA + DHA at baseline (0.55 g/d and 0.92 g/d) was associated with lower risk of fatal ischemic heart disease (IHD), but there was no association between EPA + DHA and nonfatal MI. These data are consistent with the report from a case-control study nested in the Cardiovascular Health Study *(29)*. A higher plasma concentration of EPA + DHA was associated with a lower risk of fatal IHD, but there was no association between plasma concentration of EPA + DHA and a risk of nonfatal ischemic heart disease (IHD). In a case-control study nested Västerbotten Intervention Programme (also part of the WHO's MONICA project), 78 people (cases) developed an MI, and were matched against 156 controls subjects who were randomly selected from the study. There was no correlation between fish intake or blood EPA + DHA and acute myocardial infarction (MI) *(30)*. Torres et al. *(31)* compared fish consumption in Portuguese men living in a

fishing village or rural village with IHD-related deaths. Fish consumption significantly correlated with lower IHD deaths in the fishing village setting. Meyer et al. *(32)* studied the relation between dietary fatty acids and diabetes in a prospective cohort study of 35,988 older women who initially did not have diabetes. An inverse relation between the incidence of type 2 diabetes and vegetable fat and substituting polyunsaturated fatty acids for saturated fatty acids and cholesterol was observed.

A recent food group analysis *(33)* demonstrated that red meat consumption was positively correlated with insulin and insulin sensitivity but not blood glucose in nondiabetic participants. In addition, red meat intake was positively associated with all indexes of glycemic control (i.e., blood glucose and insulin sensitivity in nondiabetic patients), after adjusting for sex, age, and BMI. In addition, a 0.52-mg/dL increase in blood glucose and a 0.3-μU/mL increase in insulin were observed for every daily serving of red meat consumed. Fish intake was also positively correlated with blood glucose, insulin, and insulin sensitivity in non-diabetic patients. However, this association became insignificant after adjustment for sex, age, and BMI of the participants.

The consensus from 17 published studies in human subjects is that CLA does not affect body weight or body composition. Some detrimental effects of the trans-10, *cis*-12 CLA isomer have also been reported in terms of altered blood lipid composition and impaired insulin sensitivity *(34)*. For example, these studies indicate that trans-10, *cis*-12 (t10,c12) conjugated linoleic acid (CLA) causes insulin resistance *(35)* and c9,t11 CLA decreases insulin sensitivity by 15% and increases 8-iso-prostaglandin F(2α) and 15-keto-dihydro-prostaglandin F(2α) excretion by 50 and 15%, respectively in obese men. These studies evidenced no effect on body composition *(36)*.

3. TYPES OF FATTY ACIDS

The amount and quality of fat in the diet may well be of importance in the development of insulin resistance and related metabolic disorders. Vessby et al. *(37)* observed that insulin sensitivity was significantly impaired on a SFA diet (–10%,) but did not change (nonsignificant) on a MUFA diet (+2%). Insulin secretion was not affected. The addition of ω-3 fatty acids influenced neither insulin sensitivity nor insulin secretion. Insulin sensitivity was 12.5% lower and 8.8% higher on the SFA and MUFA diet, respectively LDL cholesterol increased on the SFA diet (+4.1%) but decreased on the MUFA diet (–5.2), whereas lipoprotein (a) [Lp (a)] increased on a MUFA diet by 12% ($p < 0.001$). Tables 1 and 2 provide the effect of different fatty acids on glucose, lipid and other risk factors.

3.1. Saturated Fatty Acids and Insulin Resistance

In metabolic studies, different classes of saturated fatty acids (SFA) have different effects on plasma lipid and lipoprotein levels. In addition, higher levels of membrane-saturated fatty acids seem to greatly impair the action of insulin. Specifically, SFAs with between 12 and 16 carbon atoms tend to increase plasma total and LDL-C levels, whereas stearic acid does not have a cholesterol-raising effect, although may lower HDL, especially in women *(38)*, and increase Lp(a) concentrations. The evidence suggests that caproic, caprylic, and capric acids are neutral with respect to cholesterol-increasing properties and their ability to modulate LDL metabolism; lauric, myristic, and palmitic acids are approximately equivalent in their cholesterol-increasing potential, and stearic

Table 1
Effect of Fatty Acids on Glycemic Control and Lipid Profile

*Fatty acids**	*FBG*	*HbA1c*	*PPG*	*Total-C*	*HDL-C*	*LDL-C*	*TG*	*LDL/HDL*
SFA	↑	↑	↑	↑	↓	↑	↑	↑
MUFA	NC	NC	NC	↓	↑	↓	↓	↓
PUFA	NC	NC	NC	↓	↑	↓	↓	↓
n-6 FA's	NC	NC	NC	↓	↑	↓	↓	↓
n-3 FA's	NC	NC	NC	↓	↑	↓	↓	↓

*SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; n-6 FA's: Omega 6 fatty acids; n-3 FA's: Omega 3 fatty acids.

Table 2
Effect of Fatty Acids on Other Risk Factors

*Fatty acids**	*Hypertension*	*Body weight*	*Inflammatory markers*	*Dyslipidemia*	*Endothelial function*	*Antithrombotic*
SFA	↑	↑	↑	↑	↓	↑
MUFA	↓	NC	↓	↓	↑	↓
PUFA	↓	NC	↓	↓	↑	↓
n-6 FA's	↓	NC	↓	↓	↑	↓
n-3 FA's	↓	NC	↓	↓	↑	↓

*SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; n-6 FA's: Omega 6 fatty acids; n-3 FA's: Omega 3 fatty acids.

acid appears to be neutral in its cholesterol-increasing potential. Among the cholesterol-raising SFA, myristic acid appears to be more potent than lauric acid or palmitic acid, but the data are not entirely consistent. Parker et al. *(39)* examined the relationship of body mass index (BMI), abdomen-hip ratio, and dietary intake to fasting and postprandial insulin concentrations among 652 men aged 43 to 85 yr followed in the Normative Aging Study. It was observed that if saturated fatty acids as a percentage of total energy were to decrease from 14 to 8%, there would be an 18% decrease in fasting insulin and a 25% decrease in postprandial insulin.

These data suggest that overall adiposity, abdominal obesity, and a diet high in saturated fatty acids are independent predictors of both fasting and postprandial insulin concentrations. Diets high in SFA consistently impair both blood lipids *(40)* and insulin sensitivity, and because Western populations do not naturally consume diets with PUFA >15% of energy, MUFA is the ideal fatty acids with which to enrich the diet when reducing the proportion of CHO.

3.2. Trans Fatty Acids and Insulin Resistance

Trans fatty acids (TFA) are unsaturated fatty acids with at least a double trans configuration, resulting in a more rigid molecule close to a saturated fatty acid. These appear in dairy fat because of ruminal activity, and in hydrogenated oils; margarines, shortenings and baked goods contain relatively high levels of trans fatty acids. TFAs are incorporated into cell membrane phospholipids, resulting in decreased fluidity of membranes

and binding of insulin to its receptor, leading to impaired insulin action, insulin resistance, and hyperinsulinemia. In animal experiments, isomeric TFA increased LA and lowered AA concentrations in tissue phospholipids, indicating inhibition of Δ6 desaturase. Epidemiological and experimental studies suggest that a diet rich in saturated fat affects insulin sensitivity. Monoenes and dienes that have an unsaturated bond with the trans configuration (TFA) resemble saturated fatty acids with respect to structure. Louheranta et al. *(42)* evaluated the effects of diets high in TFA (TFA diet) and oleic acid (monounsaturated fat [MUFA] diet) on glucose and lipid metabolism in 14 healthy women for 4 wk. The diets provided 36.6 to 37.9% of energy (E%) as fat. In the TFA diet there were 5.1 E% trans fatty acids, and in the MUFA diet 5.2 E% oleic acid substituted for saturated fatty acids in the baseline diet. In young healthy women, the TFA diet resulted in a higher total/HDL cholesterol ratio and an elevation in triglyceride and apo B concentrations but had no effect on glucose and insulin metabolism compared with the MUFA diet. There was also no difference in the acute insulin response between the diets. In a cross-sectional study of 730 women (aged 43–69 yr) from the Nurses' Health Study I cohort, it was suggested that higher intake of trans fatty acids could adversely affect endothelial function (CRP levels were 73% higher, IL-6 levels were 17% higher, sTNFR-2 5%, E-selectin 20%, sICAM-1 10%, and sVCAM-1 levels 10% higher) and increased cardiovascular risk *(43)*. Pisabarro et al. *(44)* found a direct relationship between the intakes of TFA and type 2 diabetes in Ala carriers. Confirmation of this finding will require further research. Alstrup et al. *(45)* demonstrated that the pathological changes in insulin secretion from INS-1 cells to long-term culture with elevated levels of fatty acids are more pronounced for the cis (cis vaccenic acid and oleic acid) than the trans isomers (trans vaccenic acid and elaidic acid).

3.3. Monounsaturated Fatty Acids and Insulin Resistance

MUFAs are distinguished from the other fatty acid classes on the basis of having only one double bond. In the US, average total MUFA intake is 13 to 14% of total energy intake, an amount that is comparable to (or slightly greater than) SFA intake. In contrast, PUFAs contribute less (i.e., 7% of energy). Fanaian et al. *(46)* compared the effectiveness of a low-fat, high-carbohydrate diet (high-CHO) with a high monounsaturated fat diet (high-MUFA) using canola oil (which is high in oleic acid [18:0] and has a LA-to-ALA ratio of 2:1) on insulin resistance, serum lipids, and other variables in 48 male and female patients with T2DM (average age: 44.2 ± 0.9 yr). There were significant reductions in systolic and diastolic blood pressure and fasting plasma glucose and triglyceride concentrations, and significant increases in HDL cholesterol and insulin sensitivity in the high-MUFA group compared with the high-carbohydrate (CHO) group. The investigators concluded that after 1 yr, the MUFA enriched program was associated with a better metabolic profile in people with T2DM and that a diet high in monounsaturated fat is more likely to be followed than is a low fat, high-carbohydrate diet. These results are of special importance to vegetarians. The evidence for a relationship between MUFAs from olive oil and reduced risk of CHD were from 12 intervention studies that directly evaluated the effect of replacing MUFAs from olive oil with SFAs on serum total, LDL, and or HDL-cholesterol levels *(47,48)*. The intervention diets contained similar macronutrient profiles, (i.e., total fat, protein and carbohydrate were similar in the SFA and MUFA diets across studies) and all of the subjects were generally healthy.

Parillo et al. *(49)* demonstrated that in noninsulin-dependent diabetic patients treated with insulin, a high- MUFA diet is more effective than a high-complex-CHO diet in reducing blood glucose levels.

With the high-MUFA/low-CHO diet, a significant decrease in both postprandial glucose (8.76 ± 2.12 vs 10.08 ± 2.76 mmol/L) and plasma insulin (195.0 ± 86.4 vs 224.4 ± 75.6 pmol/L) levels was observed. Furthermore, fasting plasma triglyceride levels were reduced after the high-MUFA fat/low-CHO diet (1.16 ± 0.59 vs 1.37 ± 0.59 mmol/L). Tsihilias et al. *(50)* studied the long-term replacement of dietary monounsaturated fatty acids (MUFAs) with carbohydrate from breakfast cereals that had either a high or a low glycemic index (GI) and observed effects on blood glucose and lipids in subjects with T2DM. Changes in glycated hemoglobin, body weight, and fasting cholesterol and triglycerides did not differ significantly among groups. HDL cholesterol increased significantly, by ~10%, in the MUFA group compared with subjects who consumed either high- or low-GI cereals. The ratio of total to HDL cholesterol was significantly higher in the subjects who consumed the high-GI cereal than in the MUFA group at 3 mo but not at 6 mo. During 8-h metabolic profiles, mean plasma insulin was higher and mean free fatty acids were significantly lower in the 2 cereal groups than in the MUFA group.

Thomsen et al. *(51)* studied 16 healthy, first-degree relatives (6 men, 10 women, age [mean ± sd]: 35 ± 2 yr) with normal oral glucose tolerance tests who were evaluated for CVD risk factors. The subjects were randomized to treatment periods with either a carbohydrate-rich (CHO) diet (55 E% carbohydrate, 30 E% fat, 15 E% protein) or a diet rich in olive oil (25 E% as MUFA, 45 E% carbohydrate, 15 E% protein). The lowering effects on total cholesterol, LDL-cholesterol, triglyceride and apoB levels were found to be similar between the two diets. Slightly higher levels of HDL-cholesterol (1.4 ± 0.4 vs 1.3 ± 0.4 mmol/l, $p < 0.0001$) and apoA-1 (1.2 ± 0.3 vs 1.1 ± 0.3 mmol/l, $p < 0.05$) were found in the MUFA-diet, however. Furthermore, insulin sensitivity, as assessed by Bergman's minimal model, and the first response insulin areas were similar, as were the 24-h blood pressures and the von Willebrand Factor (vWF) levels. Insulin sensitivity index (ISI) decreased by 24% on the SFA vs MUFA diet in overweight subjects but was unchanged in lean subjects (NS). Insulin secretion was unaffected by diet, whereas total and HDL cholesterol increased significantly on the SFA diet. Subjects oxidized the least fat on the MUFA diet (26.0 ± 1.5 g/d) and the most fat on the TFA diet (31.4 ± 1.5 g/d), as is typical in healthy adults *(52)*.

Garg's *(53)* meta-analysis of 10 randomized crossover trials comparing isoenergetic high-MUFA and high-CHO diets in patients with T2DM concluded that consumption of high-MUFA diets improved fasting and postprandial blood glucose and 24-h glucose and insulin profiles while having no effect on fasting insulin and glycated hemoglobin concentrations or insulin sensitivity. It should be noted that most of the feeding trials in this meta-analysis used metabolic diets and that there were wide, unrealistic differences in total fat content between the two experimental diets in all of them, ranging from 15 to 25% of energy. In addition to Garg's report *(53)*, the results of five randomized crossover feeding trials comparing the effects of the two dietary approaches on glycemic control in diabetic patients *(54–56)* and on insulin sensitivity in subjects at high risk of diabetes *(57)* or healthy individuals have been reported.

These studies differ from those analyzed by Garg *(53)* in some important aspects: they were performed on an outpatient basis with natural foods and mostly prescribed

diets, in which olive oil was the main source of MUFA, and the difference in total fat content between diets was <15% of daily energy. In the study of Luscombe et al. *(54)*, two low-fat diets (21 and 23% energy from fat) and a high-MUFA diet (35% energy from fat) provided similar glycemic control, as assessed by fasting glucose and insulin levels, glycated hemoglobin, and 24-h urinary glucose output.

Similarly, Rodríguez-Villar et al. *(55)* reported no differences in glycated hemoglobin or in fasting and 6-h postprandial glucose and insulin profiles following a diet with 29% energy from fat and a high-MUFA diet (40% energy from fat). Another study from the same group *(56)* showed similar glycemic control in a larger cohort of diabetic patients prescribed a CHO diet and a diet high in MUFA. No differences in insulin sensitivity were found in healthy relatives of patients with diabetes *(57)* and in healthy young subjects *(58)* after a high-CHO diet and a high-MUFA diet.

Recently, Vessby et al. *(59)* performed a parallel-arm feeding trial in 162 healthy subjects who were given diets with 37% energy from fat, either a high-SFA diet (17% SFA, 14% MUFA) or a high-MUFA diet (8% SFA, 23% MUFA). Insulin sensitivity was impaired on the SFA diet but did not change on the MUFA diet, except for subjects whose relative intake of total fat was above the median of 37% energy. Taken together, these results suggest that, provided the intake of SFA is low, a MUFA diet with a total fat content of up to 40% of energy has effects on glycemic control that are similar to those of the traditional high-CHO diet with fat limited to 25 to 30% of energy.

The four most persuasive studies have evaluated when MUFAs from olive oil replaced SFAs in the diet *(33,60–62)*. These studies had small sample sizes (ranging from 21 to 41 subjects; study duration: approx 28 d), which represented a total of 117 subjects a majority of whom (82%) were young, healthy US men. There was a significant ($p < 0.05$) decrease in serum total and/or LDL-cholesterol levels. In addition, MUFAs did not decrease HDL-cholesterol. These studies provide some evidence to suggest that altering the fat composition of the diet by replacing SFAs with MUFAs from olive oil lowers serum total and LDL-cholesterol levels and has no effect on HDL-cholesterol.

Another eight studies included small sample sizes ranging 15 to 58 subjects/study *[64–71]*. There was a significant ($p < 0.05$) decrease in total and/or LDL-cholesterol levels when MUFAs from olive oil replaced SFAs in the diet in three of the eight less persuasive studies *(64–67)*. MUFAs did not alter HDL levels in the majority of these studies. West et al. *(72)* measured flow-mediated dilation (FMD) of the brachial artery, before and 4 h after 3 test meals (50 g fat, 2,615 kJ) in 18 healthy adults with T2DM. The MUFA meal contained 50 g fat from high oleic safflower and canola oils. Two additional meals were prepared by replacing 7 to 8% of MUFA with DHA and EPA from sardine oil or α-linolenic acid from canola oil. In T2DM subjects, FMD was increased 17% at 4 h vs the fasting baseline. After the MUFA meal, subjects with the largest increases in triglycerides had a decrease in FMD. In meals containing 3 to 5 g of ω-3 fatty acids, FMD was increased by 50 to 80% in high triglyceride subjects; MUFA alone had no significant effects on FMD.

In summary, the four most persuasive studies *(33,60–62)*, discussed above and which collectively represent 117 subjects, provide some evidence to suggest a relationship between replacing one fat group, SFAs, with another, MUFAs from olive oil, and reduced risk of CHD. The majority of less persuasive studies did not observe a relationship between MUFAs from olive oil and reduced risk of CHD. None of the most persuasive

or less persuasive studies suggested that MUFAs from olive oil, independent of SFA displacement, would lower serum total and LDL-cholesterol levels. Of note, on November 1, 2004, the Food and Drug Administration (FDA) *(73)* announced its approval of a qualified health claim for monounsaturated fat from olive oil and reduced risk of CHD:

> *"Limited and not conclusive scientific evidence suggests that eating about 2 tablespoons (23 grams) of olive oil daily may reduce the risk of coronary heart disease due to the monounsaturated fat in olive oil. To achieve this possible benefit, olive oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day. One serving of this product contains [x] grams of olive oil."*

3.4. Polyunsaturated Fatty Acids and Insulin Resistance

In Western diets, the ratio of ω-6 to ω-3 fatty acids ranges from 20:1 to 30:1 instead of the traditional range of between 1:1 and 12:1 *(74)*. Linoleic acid (LA, 18:2n-6) and alpha linolenic acid (ALA, 18:3n-3) and their long-chain derivatives are important components of animal and plant cell membranes. As noted earlier in the chapter, eicosanoid production is also altered by hyperinsulinism. Eicosanoids include prostaglandins and leukotrienes and are synthesised from the ω-3 and ω-6 EFAs, although those derived from ω-6 fats are more proinflammatory. Insulin stimulates Δ-5-desaturase, the enzyme responsible for the metabolism of the ω-6 EFA dihomogamma linoleic acid (DGLA) to arachidonic acid, the precursor of prostaglandin E2 (PGE2), a proinflammatory eicosanoid. ω-3 EFAs are precursors for PGE3, a noninflammatory eicosanoid. ω-6 and ω-3 EFAs compete for the Δ-5 and Δ-6 desaturase enzymes. The combination of a high ω-6 EFA intake with competition for desaturase enzyme sites, with hyperinsulism, is thus a potent stimulus for inflammation. This proinflammatory state increases the levels of cytokines (inflammatory chemicals) and free radical production *(75,76)* and thus may contribute to many autoimmune and degenerative disorders.

3.4.1. ω-6 Fatty Acids and Insulin Resistance

In humans, the ratio of ω-6 to saturated fatty acids in serum phospholipids correlates with insulin sensitivity. Because of the increased amounts of ω-6 fatty acids in the Western diet, eicosanoid metabolic products from AA—specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins—are formed in larger quantities than are the noninflammatory counterparts formed from ω-3 fatty acids, specifically EPA. The eicosanoids from AA are biologically active in small quantities and, if they are formed in large amounts, they contribute to the formation of thrombi and atheromas; the development of allergic and inflammatory disorders, particularly in susceptible people; and cell proliferation. Thus, a diet rich in ω-6 fatty acids shifts the physiologic state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time.

3.4.2. ω-3 Fatty Acids and Insulin Resistance

Antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties are beneficial effects of ω-3 fatty acids and these have been shown in secondary prevention of CHD, hypertension, type 2 diabetes, and also in some patients with renal disease, rheumatoid arthritis, ulcerative colitis, Crohn's disease, and chronic obstructive pulmonary disease. A majority of the studies were carried out with fish oils (EPA and

DHA). However, α-linolenic acid, found in green leafy vegetables, flaxseed, rapeseed, and walnuts, desaturates and elongates in the human body to EPA and DHA and, by itself, may have beneficial effects on health and in the control of chronic disease *(77)*.

ω-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer *(78)*. When humans ingest fish or fish oil, the ingested EPA (20:5n-3) and DHA (22:6n-3) partially replace the ω-6 fatty acids (especially arachidonic acid [AA; 20:4n-6]) in cell membranes, especially those of platelets, erythrocytes, neutrophils, monocytes, and liver cells. As a result, ingestion of EPA and DHA from fish or fish oil leads to: (i) decreased production of prostaglandin E2 metabolites; (ii) decreased concentrations of thromboxane A2, a potent platelet aggregator and vasoconstrictor; (iii) decreased formation of leukotriene B4, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence; (iv) increased concentrations of thromboxane A3, a weak platelet aggregator and vasoconstrictor; (v) increased concentrations of prostaglandin PGI3, leading to an overall increase in total prostaglandin by increasing PGI3 without decreasing prostacyclin PGI2 (both PGI2 and PGI3 are active vasodilators and inhibitors of platelet aggregation); and (vi) increased concentrations of leukotriene B5, which is both a weak inducer of inflammation and a chemotactic agent.

Approximately 23 studies have been conducted on the effects of ω-3 fatty acids in patients with T2DM *(79)*. In most studies, fish-oil consumption lowered serum triglyceride concentrations significantly, but in some studies plasma glucose concentrations rose. In many of these studies, however, the number of subjects was small and the dose of ω-3 fatty acids was >3 g/d and controls were lacking. Intake of 3 g ω-3 fatty acids/d decreased triglyceride concentrations significantly. At intakes of 3 g omega-3 fatty acids/d, only one study showed an increase in blood glucose concentrations.

In a randomized, double blind, placebo controlled, crossover trial, patients with T2DM consumed 6 g ω-3 fatty acids (EPA and DHA)/d for 6 mo in addition to their usual oral therapy *(79)*. Fasting serum glucose concentrations increased by 11% during the ω-3 fatty acid phase and by 8% during the placebo phase (olive oil), showing a no significant net increase of 3%. Similarly, there was no significant change in glycated hemoglobin concentrations. However, fasting triglyceride concentrations decreased by 43%, which is a highly significant change. This study is the largest and longest reported placebo-controlled trial of the effect of ω-3 fatty acids on type 2 diabetes. It showed convincingly that ω-3 fatty acid intake, along with oral therapy for diabetes, could lower triglyceride concentrations with no adverse effects on glycemic control.

Among 18 studies of type 2 diabetes or metabolic syndrome, ω-3 fatty acids had a favorable effect on triglyceride levels relative to placebo (pooled random effects estimate: –31.61; 95% confidence interval [CI], – 49.58, –13.64) but had no effect on total cholesterol, HDL cholesterol, LDL cholesterol, fasting blood sugar, or glycosylated hemoglobin, by meta-analysis. ω-3 fatty acids had no effect on plasma insulin or insulin resistance in type 2 diabetics or patients with metabolic syndrome, by qualitative analysis of four studies. Researchers at Hotel-Dieu Hospital in Paris, France conducted a study in 10 men suffering from T2DM *(80)*. Over a 4-mo period, half of the participants were given fish oil supplements (a pill form of the ω-3 fatty acid) and the other half of the participants was given a placebo. Ultimately, the group taking ω-3 supplements

experienced a decrease in both triglyceride and lipoprotein levels without harming their overall blood glucose and cholesterol levels. Toft et al. *(81)* reported that fish oil (EPA and DHA, 4 g/d for 16 wk), in doses that reduce blood pressure and lipid levels in hypertensive persons, does not adversely affect glucose metabolism. In another study *(82)*, 35 patients with T2DM complicated with hyperlipidemia and CHD were treated during 8 mo with poseidonol (an ω-3 supplement). They observed hypercoagulation and hypofibrinolysis in these patients. Treatment with poseidonol increased plasminogen activator activity and decreased coagulation and atherogenic index.

At Federico II University in Naples, Italy, researchers *(83)* divided 16 subjects with non-insulin-dependent diabetes mellitus (NIDDM) into two groups, giving half the ω-3 fatty acid supplement and the other half of the participants placebo. Again, the group given the ω-3 supplement experienced a significant decrease in triglyceride, lipoprotein, and cholesterol levels without an adverse effect on blood glucose levels. Haugaard et al. *(84)* examined the impact of a hypocaloric low-fat dietary intervention on membrane FA composition and insulin sensitivity in 21 obese individuals. They observed changes in the homeostasis model assessment-IR (HOMA-IR) correlated significantly with changes in long-chain PUFA intake ($p < 0.01$) and waist circumference ($R = 0.46$, $p < 0.05$) after 6 mo of intervention. A meta analysis *(85)* of 26 different trials on the effect of fish oil administration on both glycemic control and lipid parameters in type 2 and type 1 DM subjects showed no significant changes in glycosylated hemoglobin A1c (HbA1c) and fasting glucose levels increased with borderline significance in T2DM and significantly lower in type 1 DM. Skurnick-Minot et al. *(86)* reported a significant decrease in whole-body adiposity and adipocyte size in T2DM insulin-resistant patients after 2 mo of treatment with 1.8 g of ω-3 from fish oil capsule. A 7-yr prospective, population based, multicenter study showed that normoalbuminuria and nephropathy regression in well-controlled diabetes patients with long term diabetes (type 1 and 2) are associated with enhanced PUFA consumption and reduced saturated fatty acid intake *(87)*.

In a qualitative analysis of nine studies that assessed the effect of ω-3 fatty acids in renal disease, there were varying effects on serum creatinine and creatinine clearance and no effect on progression to end-stage renal disease. In a single study that assessed the effect on hemodialysis graft patency, graft patency was significantly better with fish oil than with placebo. No studies assessed the effects of ω-3 fatty acids on requirements for corticosteroids *(88,89)*.

Polyunsaturated fatty acids (PUFA), particularly those of the (ω-3) family, play pivotal roles as "fuel partitioners" in that they direct fatty acids away from triglyceride storage and toward oxidation, and that they enhance glucose flux to glycogen. In doing so, ω-3 PUFAs may protect against the adverse symptoms of the metabolic syndrome and reduce the risk of heart disease. ω-3s exert their beneficial effects by upregulating the expression of genes encoding proteins involved in fatty acid oxidation while simultaneously downregulating genes encoding proteins of lipid synthesis. PUFA govern oxidative gene expression by activating the transcription factor peroxisome proliferator-activated receptor-α. PUFA suppress lipogenic gene expression by reducing the nuclear abundance and DNA-binding affinity of transcription factors responsible for imparting insulin and carbohydrate control to lipogenic and glycolytic genes. In particular, PUFA suppress the nuclear abundance and expression of sterol regulatory element

binding protein-1 and reduce the DNA-binding activities of nuclear factor Y, Sp1 and, possibly, hepatic nuclear factor-4.

Collectively, the studies discussed suggest that the fuel "repartitioning" and gene expression actions of PUFA should be considered among criteria used in defining the dietary needs of (n-6) and (n-3) and in establishing the dietary ratio of (n-6) to (n-3) needed for optimum health benefit *(90)*. Recent evidence indicates that markers of inflammation are predictive of CVD *(91)* and diabetes *(92)*. Studies evaluated the direct antiatherogenic and anti-inflammatory effects of dietary (ω-3) PUFA in addition to their lipid-lowering effects. Fish oil supplementation suppresses proinflammatory cytokine production by human peripheral blood mononuclear cells (PBMC) *(93–95)* and inhibits lymphocyte proliferation *(95,96)*. Docosahexaenoic acid (DHA) results in a dose-dependent inhibition of VCAM-1 and E-selectin, and to a lesser extent, ICAM-1 gene expression in cultured endothelial cells *(97,98)*. In a recent study, Stene and Joner *(99)* reported a lower risk of type 1 DM with the use of cod liver oil during the first year of life, perhaps through the anti-inflammatory effects of long chain ω-3 fatty acids. Thus, ω-3 fatty acids appear to attenuate inflammatory responses that are important in the initiation of atherosclerosis.

People with diabetes tend to have dyslipidemia. ω-3 fatty acids from fish oil can help lower triglycerides and raise HDL, so people with diabetes may benefit in reducing dyslipidemia risk by eating foods or taking supplements that contain DHA and EPA. ALA (from flaxseed, for example) may not have the same benefit as DHA and EPA because some people with diabetes lack the ability to efficiently convert ALA to a form of ω-3 fatty acids that the body can use readily *(100)*.

Strong evidence from population-based studies suggests that ω-3 fatty acid intake (primarily from fish) helps protect against stroke caused by plaque buildup and blood clots in the arteries that lead to the brain. In fact, eating at least two servings of fish/wk can reduce the risk of stroke by as much as 50%. However, people who eat more than 3 g of ω-3 fatty acids/d (equivalent to 3 servings of fish/d) may be at an increased risk for hemorrhagic stroke, a potentially fatal type of stroke in which a blood vessel bursts in the brain or an aneurysm occurs.

Epidemiological and clinical studies also suggest that a high intake of ω-3 PUFAs protects against the development of depression. There is also some evidence that a low intake of ω-3 is associated with an increased risk of T2DM, but the results are less conclusive *(79)*. Results from randomized controlled trials in nondiabetic subjects with major depression show that EPA is an effective adjunct treatment of depression in diabetes, whereas DHA is not. Moreover, consumption of ω-3 reduces the risk of CVD and may, therefore, indirectly decrease depression in T2DM via a reduction in cardiovascular complications. Supplementation with ω-3 PUFAs, especially EPA or a combination of EPA and DHA, may be a safe and helpful tool to reduce the incidence of depression and to treat depression in T2DM *(101)*. High consumption of ω-3 FAs positively affects components of the metabolic syndrome, insulin sensitivity, and glucose tolerance. This finding suggests that high consumption of long-chain (C20-C22) ω-3 fats protects against the development of metabolic syndrome and glucose intolerance in 447 Norton Sound Eskimos (35–74 yr of age) *(102)*.

Overall, ω-3 fatty acids convey significant benefits in lowering risk of primary cardiac arrest, reducing cardiovascular mortality, particularly sudden cardiac death, reducing

triglyceride levels, increasing HDL levels, improving endothelial function, reducing excess platelet agglutination, and lowering blood pressure. However, the modest increase in LDL levels that may result from increased ω-3 intake is to be considered as a nonprotective effect on one coronary risk factor. Preliminary evidence suggests increased consumption of ω-3 fats has no effect on glucose tolerance in people with T2DM.

4. RECOMMENDATIONS

It should be considered essential to encourage a ratio of ω-6:ω-3 PUFAs of approx 1:1 to 2:1, to keep saturated fat intake low, and to limit TFA intake to ≤2% of total energy intake. EPA and DHA lower cardiovascular risk factors in people with T2DM without adversely affecting glycemic control. Hence, long-term, controlled studies are required to observe the effects of ω-3 fatty acids on metabolic risk factors in diabetes. There is a need for more research to study the changes in lipid concentrations and abnormalities in carbohydrate metabolism in people with insulin resistance and/or diabetes.

5. CONCLUSIONS

Individual fatty acids yield remarkably diverse effects on risk factors for diabetes and CVD. With respect to effects on lipids and lipoproteins, we have a reasonably good understanding of the effects of individual fatty acids. Much remains to be learned about individual fatty acids with regard to other risk factors such as diabetic risk factors, hemostatic factors, platelet function, blood pressure, and endothelial function, as well as the development of atherosclerosis and the insulin resistance syndrome. Because of the growing appreciation of the health benefits of MUFAs and PUFAs, it is likely that a mixture of these fatty acids in the diet will confer the greatest health benefits within the context of a total fat intake that is considered moderate. However, total fat needs to be considered in insulin resistance syndrome so as to not give rise to weight gain through increased percent calories from fat. Greater understanding of the roles played by dietary fat and plasma fatty acids in the pathogenesis of insulin resistance and diabetes will assist in more timely primary prevention and improved treatment in the future.

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19 Fatty Acids in the Causation and Therapy of Metabolic Syndrome

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Abstract

The role of fatty acids in the prevention and pathogenesis of metabolic syndrome leading to cardiovascular diseases, type 2 diabetes and insulin resistance are reviewed. We did Medline, PubMed search till March, 2007. Excess of linoleic acid, trans fatty acids (TFA), saturated and total fat as well as refined starches and sugar are proinflammatory. Low dietary monounsaturated fatty acids (MUFA) and n-3 fatty acids and other long chain polyunsaturated fatty acids (LCPUFA) are important in the pathogenesis of metabolic syndrome. Sedentary behaviour in conjunction with mental stress and various personality traits can enhance sympathetic activity and increase the secretion of catecholamine, cortisol and serotonin that appear to be underlying mechanisms of obesity and metabolic syndrome. Excess secretion of these neurotransmitters in conjunction of underlying long chain PUFA deficiency, and excess of proinflammatory nutrients, may damage the neurons via proinflammatory cytokines, in the ventromedial hypothalamus and insulin receptors in the brain, especially during fetal life, infancy and childhood, resulting into their dysfunction. Since 30–50% of the fatty acids in the brain are LCPUFA, especially omega-3 fatty acids, which are incorporated in the cell membrane phospholipids, it is possible that their supplementation may be protective. Omega-3 fatty acids are also known to enhance parasympathetic activity and increase the secretion of anti-inflammatory cytokines IL-4 and IL-10, as well as acetylecholine in the hippocampus. It is possible that marginal deficiency of LCPUFA, especially n-3 fatty acids, due to poor dietary intake during the critical period of brain growth and development in the fetus and infant, and also possibly in the child, adolescents and adults, may enhance oxidative stress and the release of proinflammatory cytokines; tumor necrosis factor-alpha, interleukin-1, 2 and 6 and cause neuronal and beta-cell dysfunction. Experimental studies indicate that ventromedial hypothalamic lesion in rats induces hyperphagia, resulting in glucose intolerance and insulin resistance. Administration of neuropeptide Y abolished the hyperphagia and ob mRNA (leptin mRNA) in these rats. Treatment with diets rich in MUFA and omega-3 fatty acids, meditation, beta blockers, ACE inhibitors, and phytochemicals may have a beneficial influence on insulin receptors and ventromedial hypothalamic dysfunction, causing beneficial effects in metabolic syndrome. Despite weaknesses, epidemiological studies and intervention trials indicate that treatment with n-3 fatty acids and MUFA rich foods may be applied to clinical practice and used to direct therapy for prevention of metabolic syndrome. Intervention trials with Columbus diet and lifestyle in patients with metabolic syndrome would be necessary to provide a proof for our statement.

Key Words: Insulin resistance; brain disease; diet nutrition; n-3 fatty acids; brain development; brain-heart connection; monounsaturated fatty acids; polyunsaturated fatty acids.

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1. INTRODUCTION

Insulin resistance is the underlying pathophysiological defect in the metabolic syndrome which is characterized with cluster of specific abnormalities *(1,2)*. The clinical diagnosis of metabolic syndrome is based on measures of abdominal obesity, atherogenic dyslipidemia, and raised blood pressure and glucose intolerance *(1–3)*. About one-fifth of the adult population in developing countries and one-forth in industrialized countries, may have metabolic syndrome *(4–6)*. Dietary intake of total fat amounts to between 25 and 45% in developed countries and 15 and 35% of total energy in developing countries *(3–13)*. Most of the dietary fatty acids are derived from meats, oils and dairy products, resulting in to marked increase in saturated and ω-6 fatty acids but relatively modest of monounsaturated fatty acids (MUFAs) and long-chain polyunsaturated fatty acids (PUFAs) *(10)* particularly ω-3 fatty acids. Refining of vegetable oils has been a major cause for increased consumption of ω-6 fatty acids and hydrogenation of these oils caused greater intake of trans fatty acids, that may be the cause of increased prevalence of metabolic syndrome, in most countries of the world *(12–16)*.

Indians were aware about the adverse effects of the diet from the ancient times, which is evident from the following verse from an ancient scripture Bhagwatgeeta. "Foods which are bitter, acid, salted, burnt, fried and pungent, give rise to pain, mental stress and diseases" (3100 BCE). In the 7th century BCE, one Chinese physician proposed that "increased consumption of salt may cause hardening of the pulse." Charaka and Sushruta (600 BCE), two great physicians of India, knew about the role of diet and lifestyle in the pathogenesis of heart attack, which would be clear from the following verse. "Heart attack is born by the intake of fatty meals, overeating, excess of sleep, lack of exercise and anxiety." Charaka Sutra, 600 BCE.

About 2000 yr ago from now (1st century), Confucius, the Chinese philosopher taught his students, "the higher the quality of foods, the better and never rely upon the delicacy of cooking." Thus a dietary guideline based on experience, observation, and thinking was given as, "cereals, the basic, fruits the subsidiary, meat the beneficial and vegetable the supplementary." Therefore, the concept of eating a diet high in animal foods and a preference for meat and greasy foods have been shaped over hundreds of years among Chinese.

However, these dietary patterns were associated with enormous physical activity and sports such as hunting and also possibly meditation as a result of the introduction of Buddhism, possibly causing no significant problem of noncommunicable diseases during that period. Moreover, the meat may have been from running animals, meaning it contained useful fatty acid composition. It appears that the Columbus® diet and lifestyle was known to Indians and Chinese from the ancient period. The Columbus concept of diet means that humans evolved on a diet that was low in saturated fat and the amount of ω-3 and ω-6 fatty acids was quite equal. There have been marked changes in the food supply resulting from the development of agriculture over the last 10,000 yr ago. However, it was only during the last 100 to 150 yr that dietary intakes changed significantly, causing increased intake of saturated fatty acids (SFA) from grain fed cattle, tamed at farm houses, rather than meat from running animals *(11–15)*.

2. METABOLIC SYNDROME

A clustering of risk factors occurrs with overweight and central obesity, which may be associated with impaired glucose tolerance, an adverse lipid profile, and hypertension;

effects may be seen as early as childhood and adolescence *(1–3)*. These risk factors, which are indicator of metabolic syndrome, also tend to be clustered in children and adolescents with unhealthy lifestyles and diets such as those with excessive intakes of saturated fats, cholesterol, refined carbohydrates and salt, and inadequate consumption of dietary fiber, antioxidants, vitamins, minerals, and ω-3 fatty acids. Lack of physical activity and increased television viewing are other factors that further increase the risk *(1–3)*. In older children and adolescents, habitual alcohol and tobacco use also contributes to high blood pressure and to the development of other risk factors in early adulthood that affect them later in life. This clustering of risk factors represents an opportunityto address more than one risk factor at a time and may result from the clustering of health related behaviors (Table 1).

Of the several characteristics of metabolic syndrome, at least three should be present for its diagnosis. Obesity in conjunction with type 2 diabetes, hypertension, coronary artery disease (CAD), and dyslipidemia are important features of the metabolic syndrome which is usually associated with hyperinsulinemia and insulin resistance. Several names have been given by various investigators *(1,2)* for this syndrome: insulin resistance syndrome, Reaven's syndrome, deadly quartet, CHAOS, new world syndrome, civilization syndrome, syndrome X, and finally metabolic syndrome which is also now accepted by the World Health Organization (WHO).

3. NUTRITION IN TRANSITION AND METABOLIC SYNDROME

There is coexistence of nutritional deficiencies and appreciable overnutrition in the form of central obesity and overweight in both developed and developing countries *(1–16)*. The Global Burden of Disease Study clearly showed that the gains in cardiovascular health occurred in developed countries, in association with an epidemic of CVD in the developing world *(12–14)*. We have been in a position to study the mechanism of transition from poverty to economic development and the emergence of CVD. It seems that metabolic syndrome is an important pathway for development of CVD and type 2 diabetes. We proposed that "overweight comes first in conjunction with hyperinsulinemia, increased angiotensin activity, increased proinflammatory cytokines, and central obesity followed by glucose intolerance, type 2 diabetes, hypertension, low high-density lipoprotein cholesterol (HDL), and hypertriglyceridemia (Metabolic syndrome). This sequence is followed by CAD, gall stones, and cancers and finally dental caries, gastrointestinal diseases, and bone and joint diseases, during transition from poverty to affluence *(3–5)*." As people become rich, they begin to increase their dietary fat, salt, and sugar (proinflammatory foods) intake in the form of ready prepared foods, syrups, dairy products, and flesh foods in place of grain based diet. There is a greater use of automobiles, television viewing, and a decrease in sports, walking, and dancing as recreation. These changes in the diet and lifestyle in conjunction with increased tobacco and alcohol intake, appear to be basic factors in the pathogenesis of noncommunicable diseases, including metabolic syndrome *(3,5)*. The last few decades of the 20th century have offered us an opportunity to initiate action to counter growing epidemics of CVD including metabolic syndrome on both sides of the Atlantic. When people learned the methods of prevention, there was a decrease in CVD in the Western world but obesity continued to increase, resulting into an increase in the metabolic syndrome in both developed and developing economies *(1–9)*.

Table 1
Causes and Components of Metabolic Syndrome

Diet
Excess of saturated fat.
Excess of trans fatty acids
Excess of ω-6 fatty acids
Excess of refined starch and sugar
Lack of phytochemicals.
Lack of ω-3 fatty acids and
Monounsaturated fatty acids.
Lifestyle
Cigarette smoking
Sedentary behavior
Lipoproteins
Increased apo B
Decreased apo A-1
Decreased HDL
Increased small dense LDL
Increased apo C-III
Prothrombotic
Increased angiotensin
Increased fibrinogen
Increased plasminogen activator inhibitor 1
Increased viscosity
Inflammatory markers
Increased IL-6
Increased TNF-α
Increased resistin
Increased C-reactive protein
Decreased adiponectin
Increased white blood cell count
Decreased anti-inflammatory Interleukin-10
Vascular
Decreased endothelial function
Microalbuminuria
Increased asymmetric dimethylarginine
Other
Increased uric acid
Increased homocysteine
Non-alcoholic steatohepatitis
Polycystic ovaries syndrome
Obstructive sleep apnoea

There has been an enormous increase in ω-6 fatty acid (about 30 g/d) in the diet resulting from the production of oils from vegetable seeds such as corn, sunflower, soybean, and cotton. Increased intake of meat has resulted into greater intake of arachidonic acid (0.2–1.0 mg/d), whereas the consumption of α-linolenic acid (ALA) has decreased

Table 2
Effect of ω-3 Fatty Acid Rich Diet in Patients With Acute Myocardial Infarction *(87–89)*

	Intervention group (n = 204)		*Control group (n = 202)*	
Foods and nutrients	*4–7 d*	*After 1 yr*	*4–7 d*	*After 1 yr*
Fruits and vegetables (g/d)	508.4 ± 28.66[a]	575 ± 91.4[a]	254.4 ± 17.2	220.5 ± 19.6
Legumes and pulses (g/d)	80.5 ± 6.6[a]	95.0 ± 8.9[a]	52.5 ± 4.6	45.6 ± 5.6
Almonds and walnuts (g/d)	82.4 ± 5.7[a]	75.5 ± 5.2[a]	–	–
Fish (g/d)	52.5 ± 6.5[a]	22.4 ± 4.1[a]	20.2 ± 3.1	10.5 ± 3.5
Chicken (g/d)	–	10.2 ± 3.2[b]	76.2 ± 6.5	66.5 ± 10.5
Mustard or soybean oil	18.4 ± 3.9[b]	31.5 ± 5.5[a]	10.5 ± 2.3	6.8 ± 2.8
Butter or clarified butter (g/d)	2.5 ± 0.6[a]	3.3 ± 0.71[b]	10 ± 2.6	12.6 ± 3.5
Skim milk (mL/d)	161.2 ± 12.0	152 ± 14.5[b]	150.2 ± 8.0	165.5 ± 16.1
Wheat chapati	5.5 ± 1.6[a]	30.6 ± 5.5	50.6 ± 6.6	55.6 ± 7.8
Bread, biscuits (g/d)	10.6 ± 2.2[b]	25.5 ± 6.2[a]	230.6 ± 20.1	212.2 ± 18.1
Rice and wheat cereals (g/d)	25.6 ± 2.4	30.6 ± 5.5	30.2 ± 3.1	35.6 ± 4.8
Honey or raisins (g/d)	2.6 ± 0.8	5.5 ± 1.2	–	–
Sugar (g/d)	16.4 ± 3.7[b]	12.6 ± 3.4[b]	25.5 ± 5.4	30.5 ± 7.6
Alpha-linolenic acid (g/d)	1.0 ± 0.46[a]	1.8 ± 0.66[a]	0.5 ± 0.11	0.65 ± 0.14
Ascorbic acid (mg/d)	474 ± 70.5[a]	440.6 ± 100.6[a]	150.2 ± 10.6	160 ± 38.0

Notes: p values were obtained by comparison between intervention and control groups after 1 wk and after 1 yr.
[a] = $p < 0.01$.
[b] = $p < 0.05$.

(about 0.55 g/d) and the amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are 48 and 72 mg/d, respectively *(8–11)*. A relative and absolute decrease in ω-3 fatty acids has led to an imbalance and increase in the ratio of ω-6:ω-3 fatty acids to up to 50 in India and other developing countries, consuming vegetable seed oils (e.g., corn, soybean, sunflower, cotton). Saturated fatty acids (SFA) and trans fatty acids (TFA) elevate, PUFAs decrease, and MUFAs have beneficial effects on total and low-density lipoprotein cholesterol (LDL) as well as on HDL cholesterol. ω-6 PUFAs and TFAs also decrease HDL cholesterol and increase insulin resistance, free radical stress, and inflammation, which may enhance atherosclerosis *(12–17)*. Increased intake of total fat, TFA, SFA and ω-6 fatty acids and refined carbohydrates, may cause insulin resistance resulting into metabolic syndrome *(3–11)*. Decreased intake of MUFA, ω-3 fatty acids, fiber and phytochemicals, may enhance the metabolic syndrome.

4. METABOLIC SYNDROME IN SOUTH ASIANS

Coronary risk factors in South Asians include low concentration of HDL cholesterol, hypertriglyceridemia, abdominal obesity, high prevalence of type 2 diabetes, and CAD,

indicating presence of metabolic syndrome *(3–9)*. Sedentary lifestyle, an increase in dietary fat and refined carbohydrates, and increases in alcohol consumption are common behavior patterns underlying above risk factors *(1,2)*. Insulin resistance is nearly universal in all these conditions and South Asians are at the greatest risk of developing metabolic syndrome *(3–9)*. These behavior patterns result in hyperinsulinemia although insulin resistance differs and may not occur in all tissues of the body. Adipose tissue is not resistant to insulin in the early stages of whole-body insulin resistance *(9)* but muscle is resistant very early in the progression of metabolic syndrome X. Therefore, physical activity and yogasans appear to be important in the prevention and treatment of insulin resistance. There is no scientific evidence to demonstrate that metabolic syndrome among South Asians is genetic in origin. It is possible that populations in developing countries, under scarcity, may have adapted to survive at low-fat intake and physically demanding occupations, which made them more susceptible to dietary energy, sedentary behavior, and to established risk factor *(4,5)*. Abdominal obesity (central deposition of fat) in South Asians appears to be universal in all the countries, wherever they are living, but whether the development of this type of obesity is genetic, dietary, caused by low physical activity, or a combination of these factor has not been established *(5,9)*. Central obesity appears to be more prominent in the South Asian population than in the Western population.

Das has reviewed and suggested that transgenic mice overexpressing 11β hydroxysteroid dehydrogenase type 1 (11βHSD-1) selectively in adipose tissue can develop abdominal obesity and exhibit insulin-resistant diabetes, hyperlipidemia and hyperphagia despite hyperleptinemia *(9)*. It is not known whether adipose tissue from the abdomens of South Asians show increased 11βHSD-1 activity. If this is true, peroxisome proliferator-activated receptor-γ ligands, which markedly reduce adipocyte 11βHSD-1 activity in vitro and in vivo preferentially reduce abdominal fat, may be the drugs of choice in South Asians to reduce insulin resistance and obesity *(9)*. South Asians may be genetically programmed or suffering from maladaptation which results in the overexpression of 11βHSD-1 in their adipose tissue; this may account for their higher risk of metabolic syndrome. It is possible that treatment of central obesity with these agents may be protective against metabolic syndrome in South Asians.

Singh et al. *(4)*, in one cross-sectional survey of 255 rural 311 urban elderly subjects showed that mean blood pressures, body mass index (BMI), insulin levels, and the prevalence of hypertension were significantly greater among urban compared with rural populations. Total fat intake was significantly greater among urban and hypertensive subjects compared with rural normotensive and hypertensive subjects, respectively. A recent study *(6)* of 54 patients of acute myocardial infarction (AMI) showed that the intake of large meals and large breakfast >1000 cal especially rich in TFA was significantly associated with AMI compared with control subjects. Those consuming large meals showed significantly greater levels of tumor necrosis factor-α(TNF-α) and interleukin-6 (IL-6) compared with subjects consuming a smaller meal. In another study *(7)* of 202 patients, large meals were a trigger for the development of AMI among 50% of the patients. TNF-α and IL-6, incidence of known hypertension, and type-2 diabetes were significantly greater among AMI patients compared with healthy subjects. These proinflammatory markers are risk factors of AMI and metabolic syndrome *(3,9)*. In one cross-sectional survey, of 3257 women, aged 25 to

Table 3
Effect of Dietary Intervention on Oxidative Stress, Vitamins, and Cardiac Damage *(87)*

	Intervention group (n = 204)		*Control group (n = 202)*	
	Baseline	*Day 7*	*Baseline*	*Day 7*
Plasma ascorbic acids (23.2 ± 3.2, μmol)	7.38 ± 1.7	30.6 ± 4.7[a]	7.9 ± 1.7	15.8 ± 3.8
Plasma lipid peroxides (1.4 ± 0.41 pmol/1)	3.56 ± 0.71	2.9 ± 0.62[b]	3.48 ± 0.7	3.38 ± 0.62
Lactate dehydrogenase (216.5 ± 36.5 IU/1)	228.8 ± 48.6	656.5 ± 75.6[b]	224.2 ± 45.4	785.4 ± 81.2

Notes: p value was obtained by comparison of intervention and control groups on the day 7 after onset of symptoms of myocardial infarction.Values are mean + SD.

[a] = $p < 0.01$.

[b] = $p < 0.05$.

64 yr, social classes (1–3) were consuming significantly greater amounts of total visible fat—including TFA, clarified butter (Indian ghee), and vegetable oils—as compared with lower social classes (4 and 5). Mean BMI, overweight (BMI > 25K g/M^2), and central obesity (Waist-hip ratio > 85) were significantly greater among the higher rather than the lower social classes *(8)*.

Higher social classes (1–3) are known to have greater prevalence of CAD, type 2 diabetes, and hypertension, indicating metabolic syndrome in Indians. It is possible that lower intake of ω-3 fatty acids and MUFAs, as well as increased consumption of ω-6 fatty acids and TFAs may be responsible for central obesity and metabolic syndrome among these patients *(8–12)*. In another study *(13)*, of 850 men, 25 to 64 yr of age, subjects were divided into high fat, over-fat, normal-fat, and under fat based on criteria of body fat analysis by bioelectrical impedance. The prevalence of CAD, type 2 diabetes, and hypertension, as well as low-HDL cholesterol, BMI, and WHR were significantly associated with high body fat percent.

5. GENE AND ENVIRONMENT INTERACTIONS AND METABOLIC SYNDROME

Dietary factors and physical activity, mental stress and environmental toxicants can influence gene expression and have shaped the genome over several million years of human evolution *(5,11)*. There is opportunity for health, as well as susceptibility to diseases, through genes, although environmental factors determine which susceptible individuals will develop metabolic syndrome. Rapid changes in diet and lifestyle resulting from socioeconomic changes provide added stress, causing exposure of underlying genetic predisposition to chronic diseases such as type 2 diabetes, obesity, hypertension, CAD, and atherosclerosis. Several studies are continuing on the role of nutrients in gene expression *(11)*. It is not clear how n-3 fatty acids suppress or decrease the mRNA of interleukin, which is elevated in atherosclerosis, arthritis, and other autoimmune diseases; whereas n-6 fatty acids have no such effects *(11)*. Metabolic syndrome appears to be polygenic in nature and rapidly escalating rates suggest the importance of environmental change, rather than changes in genetic susceptibility.

Table 4
Effect of Cardioprotective Diet on Cardiac Events After 1 yr *(89)*

	Intervention group (n = 204)	*Control group (n = 202)*
Non-fatal myocardial infarction	30 (15)[a]	48 (24)
Fatal myocardial infarction	13 (6)[b]	19 (9)
Sudden cardiac death (<1h)	7 (3)[b]	15 (7)
Suspected cardiac death	–	1 (0.5)
Death due to stroke	1 (0.5)	2 (1.0)
Death due to cancer	–	1 (0.5)
Total cardiac mortality	20 (10)[a]	34 (17)
Total mortality	21 (10)[a]	38 (19)
Total cardiac events and deaths	51 (25)[a]	86 (43)

Notes: p value was obtained by comparison of intervention and control group by a *Z*-test for proportions.
[a] = $p < 0.01$.
[b] = $p < 0.05$.

It has been proposed *(3–14)* that genetic and other factors including oxidative stress, superoxide anion (O2) and hydrogen peroxide (H2O2), endothelial nitric oxide (eNO), lipid peroxides, anti-oxidants, endothelin, angiotensin converting enzyme (ACE) activity, angiotensin-II, transforming growth factor-β (TGF-β), insulin, homocysteine, asymmetrical dimethyl arginine, proinflammatory cytokines, IL-6, TNF-α, C-reactive protein (hs-CRP), long-chain polyunsaturated fatty acids (LCPUFAs), and activity of NAD(P)H oxidase all have a role in human essential hypertension. There is a close interaction between endogenous molecules (e NO, endothelin, cytokines) and nutrients, (folic acid, coenzyme Q10, L-carnitine, L-arginine, tetrahydrobiopterin (H4B), vitamin B6, vitamin B12, vitamin C, and LCPUFAs). Statins can mediate some of their actions through (LCPUFAs), whereas these fatty acids (especially ω-3 fatty acids) suppress cyclo-oxygenase activity and the synthesis of proinflammatory cytokines, and activate parasympathetic nervous system. This activity can reduce the risk of major vascular events. LCPUFAs such as EPA, DHA from precursors to lipoxins and resolvins may have anti-inflammatory actions. Low-grade systemic inflammation seen in hypertension seems to have its origins in the perinatal period and availability of adequate amounts of LCPUFAs during the critical periods of brain growth prevents the development of hypertension *(3,12)*. This indicates that preventive strategies aimed at decreasing the incidence of hypertension and its associated conditions such as atherosclerosis, type 2 diabetes, CAD and cardiac failure in adulthood need to be prevented during the perinatal period for primordial prevention of metabolic syndrome.

6. DIETARY FATTY ACIDS AND METABOLIC SYNDROME

Experimental studies indicate that type and amount of dietary fatty acids may cause insulin resistance and metabolic syndrome *(17)*. MUFA or ω-3 fatty acids appear to have beneficial effects on insulin action, whereas ω-6 fatty acids, saturated fats, and diets with high total-fat content, appear to decrease insulin sensitivity in animal studies

Table 5
Therapeutic Goals and Recommendations of the International College of Cardiology for Management of Metabolic Syndrome

Abdominal obesity Goal: 10% weight loss first year, thereafter continued weight loss or maintain weight.
Recommendation: caloric restriction; regular exercise; behavior modification.
Physical inactivity goal: regular moderate-intensity physical activity.
Recommendation: 30–60 min moderate to intense exercise daily.
Atherogenic diet goals: reduced intakes of saturated fats (7%), no TFAs.
Recommendations: saturated fat, 7% of total calories; no TFAs; ω-6:ω-3 ratio <0.5 mono unsaturated fat 10–15% energy, dietary cholesterol, <100 mg daily; total fat 20–30% of total calories.
Fruits, vegetables (400 g/d), almonds, walnuts (50 g/d), whole grains, and legumes (400 g/d), olive oil + lintseed oil (Columbus oil), 25–50 g/d.
Cigarette smoking goal and recommendation: complete smoking cessation
LDL-C Goals: High-risk patients[a]—LDL cholesterol 1 g/L (2 · 6 mmol/L)
Therapeutic option—LDL cholesterol 0 · 7 g/L (1 · 8 mmol/L)
Moderately high-risk patients[b]—LDL cholesterol 1 · 3 g/L (3 · 4 mmol/L)
Therapeutic option—LDL cholesterol 1 g/L (2 · 6 mmol/L)
Moderate-risk patients[c]—LDL cholesterol 1 · 3 g/L (3 · 4 mmol/L)
Recommendations: high-risk patients—lifestyle therapies[d] and LDL-cholesterol lowering drug to achieve recommended goal
Moderately high-risk patients—lifestyle therapies; add LDL-cholesterol lowering drug if necessary to achieve recommended goal when baseline LDL cholesterol 1 · 3 g/L (3 · 4 mmol/L)
Moderate risk patients—lifestyle therapies; add LDL-cholesterol lowering drug if necessary to achieve recommended goal when baseline LDL cholesterol 1 · 6 g/L (4 · 1 mmol/L)
High triglyceride or Goal: insufficient data to establish goal low-HDL-C Recommendation: High-risk patients—consider adding fibrates (preferably fenofibrates) or nicotinic acid to LDL-lowering drug therapy
Elevated blood pressure Goals: blood pressure 135/85 mmHg. For diabetes or chronic kidney disease: blood pressure 130/80 mmHg
Recommendation: lifestyle therapies; add antihypertensive drug(s) when necessary to achieve goals of therapy, preferably ACE inhibitors or metaprolol.
Elevated glucose goal: maintenance or reduction in fasting glucose if 1 g/L (5 · 5 mmol/L). Haemoglobin A1C 7 · 0% for diabetes
Recommendation: lifestyle therapies; add hypoglycaemic agents as necessary to achieve goal fasting glucose or haemoglobin A1C
Prothrombotic state Goal: reduction of prothrombotic state
Columbus soup (tomato, grapes, vegetables, almonds, walnuts, lint seed + olive oil), Columbus yogurt (walnut, almonds, raisins) in patients with acute coronary syndromes.
Recommendation: High-risk patients—initiate low-dose aspirin therapy; consider clopidogrel if aspirin is contraindicated.
Moderately high-risk patients—consider low-dose aspirin therapy.
Proinflaammatory state recommendations: give ω-3 fatty acids, and statins, fibrates, and pioglitazones to be considered.

[a]High-risk patients: those with established atherosclerotic cardiovascular disease, diabetes, or 10-yr risk for coronary heart disease 20%.
[b]Moderately high-risk patients: those with 10-yr risk for CHD 10–20%.
[c]Moderate risk patients: those with metabolic syndrome but 10-yr risk for CHD 10%.
[d]Lifestyle therapies include weight reduction, regular exercise, and antiatherogenic diet.
Modified from refs. *1–5*.

(17–19). It is hypothesized that dietary fats affect the phospholipid composition of cell membranes in skeletal muscle and other tissues. Several clinical studies showed a decrease in insulin sensitivity with high fat diets *(20,21)*.

A few studies diminish the strength of their conclusions, including large difference in diets, the nonrandomized assignment of diets and lack of standardized methods to measure insulin sensitivity. Other studies using more standard measures reported a relationship between fat content and insulin sensitivity *(22)*. One reason may be the relatively short duration of intervention in many of these studies. A recent multicenter, 3-mo investigation found that a diet high in saturated fat (18% of energy) decreased insulin sensitivity more than a diet high in monounsaturated fat (21% of energy) among 162 healthy men and women *(23)*. Many cross-sectional epidemiologic studies also demonstrated positive association between intake of saturated fat and hyperinsulinemia, after adjustment for measure of body fat *(24,25)*. At least one large, well-designed study showed no association *(26)*. Prospective studies including the Nurses' Health Study *(27)* suggest the role of specific types of fat in the development of type 2 diabetes mellitus. In the Nurses' Health Study, investigators reported an inverse association between development of diabetes and intake of vegetable fat and PUFAs, a positive association for *TFAs,* but no association for total fat in the diet. In a more recent study *(28)* of 15 obese, hyperinsulinemic subjects, a hypoenergetic MUFA-rich diet containing 35% carbohydrate, 45% fat, and 20% protein was compared with a diet containing 60% carbohydrate, 20% fat, and 20% protein. After 4 wk fasting insulin levels, insulin to glucose ratio, and Homeostatic Model and Assessment Index decreased to normal ranges and were significantly lower in high-MUFA group as compared with the control group. However, insulin sensitivity score increased significantly more and waist circumference showed significant decline in the high-MUFA group compared with the high-carbohydrate group, indicating improved insulin sensitivity and decreased central obesity, respectively, with a MUFA-rich diet. In a cross-sectional population study *(29)*, Folsom et al. reported on 4000 healthy subjects, finding that fasting insulin levels were positively associated with the percentage of saturated fat in plasma and inversely associated with persentage of MUFA. Lovejoy et al. *(30)* observed that certain class of fatty acids such as ω-6, saturated, and TFA have more deleterious effects on insulin action than others, and increase the risk of type 2 diabetes mellitus and therefore metabolic syndrome. Low and coworkers *(31)* found that a high-MUFA diet induced improvement in the control of type 2 diabetes as compared with high carbohydrate diet.

Similar results were noted by other researchers *(28,32)*. In one study, a MUFA-rich diet also reduced the total cholesterol to HDL-cholesterol ratio compared with high carbohydrate diet *(33)*.

A greater reduction in triglyceride levels was observed in the high MUFA group than in the high carbohydrate group *(33,34).* These results were observed in earlier studies *(34)* showing that dietary fatty acid composition affects the fatty acid composition of VLDL-triglyceride and alterations in composition of VLDL and in the enzymes involved in its catabolism, are two mechanisms observed in the hypotriglyceridemic effect of high-MUFA diets *(35)*. Oleic acid has also been found to cause a reduction in triglyceride levels, possibly through increasing the removal of triacylglycerol *(36)*. Moreover, olive oil has been found to promote gastrointestinal secretions and stimulate stomach emptying thereby increasing the rate of supply of fatty acids to the enterocytes

(37), thus accelerating the rate of digestion and absorption and faster rate of entry of chylomicrons into the circulation. This implies that the long-term use of olive oil may cause upregulation of the enterocytes' ability to process dietary triacylglycerol and synthesize chylomicrons *(38)*. However, high carbohydrate diets are known for their hypertriglyceridemic effects and glucose intolerance *(39)* which appear to result from downregulation of muscle lipoprotein lipase (LPL) activity *(40)*. These adverse effects of such diets can enhance diabetes and cardiovascular disease or metabolic syndrome *(41–45)*. The Columbus® concept of diet; including fruits, vegetables, whole grains, nuts, ω-3 fatty acid rich eggs and meats, and Columbus oil (olive oil + lint seed oil) in conjunction with physical activity addresses both diet and lifestyle, and may be useful in the prevention of metabolic syndrome as well as its components *(16,46–48)*.

7. NUTRITION AND INFLAMMATION

Some indications that vascular indexes should be independently considered as risk factors for atherothrombosis are anti-inflammatory effects of statins, hormone replacement therapy (HRT), and post-prandial endothelial dysfunction in relation to inflammation *(49,50)*. Impaired vascular biology, physiology, and biochemistry resulting into inflammation and endothelial dysfunction may be independently atherothrombotic *(49–51)*. There is evidence that abnormalities of the post-prandial state are important contributing factors to the development of atherosclerosis *(6,7,49–51)*. Recent studies indicate that changes in LDL cholesterol and C-reactive protein independently correlate with coronary atherosclerosis progression and coronary events *(52,53)*. On treatment, however, C-reactive protein was as predictive of subsequent coronary events as was LDL cholesterol; HRT increases HDL cholesterol and endothelial function, as well as inflammation and coagulation which are atherogenic. HRT was therefore discarded.

Clinical data indicate that post-prandial hypertriglyceridaemia is a risk factor for cardiovascular disease in nondiabetic subjects and may be a predictor of carotid intima-media thickness in type 2 diabetic patients *(54)*. Meal absorption is a complex phenomenon, and post-prandial hyperlipidaemia, and hyperglycemia are simultaneously present in the post-absorptive phase, particularly in diabetic patients or in subjects with impaired glucose tolerance *(49,51,54)*. Both post-prandial hyperglycemia and hypertriglyceridaemia may cause endothelial dyfunction, which is considered an early marker of atherosclerosis *(49,50)*. The effect of different isocaloric meals on endothelial function in both normal subjects and type 2 diabetic patients may be that the level of triglycerides after a high-fat (saturated) meal are associated with endothelial dysfunction, with maximal impairment occurring at the time of the simultaneous presence of post-prandial hyperglycemia and hypertriglyceridaemia *(49,50)*. The effect of liquid meals rich in carbohydrates or saturated fats may be similar *(49)*. It is possible that endothelial dysfunction is induced by high-fat meals in type 2 diabetic patients or associated with fasting hypertriglyceridaemia in young men and could be associated with increased plasma concentrations of asymmetric dimethylarginine, an endogenous nitric oxide synthase inhibitor, suggested as a novel cardiovascular risk factor *(56)*.

Further proof for the Columbus® concept was provided in more recent studies on the effect of meals on risk factors of atherothrombosis. Consumption of high-fat meals and vegetable foods rich in natural antioxidants largely prevents the negative effects on endothelial function *(57)*. In particular, endothelial dysfunction acutely

triggered by the consumption of a high-fat meals rich in saturated fatty acids is reduced by the simultaneous consumption of a vegetable serving including pepper (100 g) tomatoes (100 g), and carrots (200 g). A mild pro-oxidative state accompanies meal ingestion, which results in raised circulating biomarkers of inflammation, adhesion, and endothelial dysfunction, all of which are factors in the development of cardiovascular disease *(58)*. The effect of hyperglicaemia, hypertriglyceridaemia, and raised FFA levels, both fasting and post-prandial, on endothelial function may be mediated through the generation of an oxidative stress *(49,50,57)*.The process is supposed to involve increased super-oxide generation, which in turn inactivates nitric oxide. Superoxide and nitric oxide combine to produce peroxynitrite, a potent and long-lived oxidant that is cytotoxic, initiates lipid peroxidation, and nitrates amino acids such as tyrosine that affect many signal transduction pathways. The production of the peroxynitrite anion can be indirectly inferred by the presence of nitrotyrosine (NT). An increase in plasma NT levels has been reported in association with post-prandial hyperglycemia or hypertriglycidemia, with a cumulative effect occurring in the presence of both conditions *(49)*. It seems therefore that oxidative stress is a mediator of the effect of raised substrate concentration in the post-prandial phase *(59)*. It is clear that what happens during the absorption phase may be of considerable importance because it occurs several times each day and human beings now spend an increasingly greater part of their lives in the post-prandial phase without periods of fasting. These biological markers after high-fat meals also rich in refined carbohydrates appear to be basic underlying mechanisms for insulin resistance and metabolic syndrome *(60)* (Figs. 1,2).

8. PROINFLAMMATORY MACRONUTRIENTS

Proinflammatory macronutrients such as ω-6 fatty acids, TFA, and SFA, as well as refined carbohydrates intake may produce oxidative stress and proinflammatory substances *(49,50)* (Fig. 1). Glucose ingestion in normal subjects is associated with increased superoxide generation in leukocytes and mononuclear cells, as well as with raised amount and activity of nuclear factor-κB (NF-κB), a transcriptional factor regulating the activity of at least 125 genes, most of which are pro-inflammatory *(60)*. Increased consumption of refined carbohydrates also causes an increase in two other pro-inflammatory transcription factors: (i) activating protein-1(AP-1) and (ii) Egr-1. The first regulated the transcription of matrix metalloproteinases and the second modulates the transcription of tissue factor and plasminogen activator inhibitor-1 *(49,60)*. A mixed meal from a fast-food chain has also been shown to induce activation of NF-κB associated with the generation of reactive oxygen species (ROS) by mononuclear cells (Fig. 1). Superoxide anion appears to be an activator of at least two major pro-inflammatory transcription factors, NF-κB and AP-1. These observations are consistent with previous findings, demonstrating that after oral or intravenous glucose challenges, in both normal subjects and patients with type 2 diabetes mellitus, there is an increased generation of ROS and raised circulating levels of proinflammatory cytokines, such as TNF-α, IL-6, and IL-18 *(49,50,61–63)*. In apparently healthy subjects, a single high-fat meal produced endothelial activation, as evidenced by increased concentrations of the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion

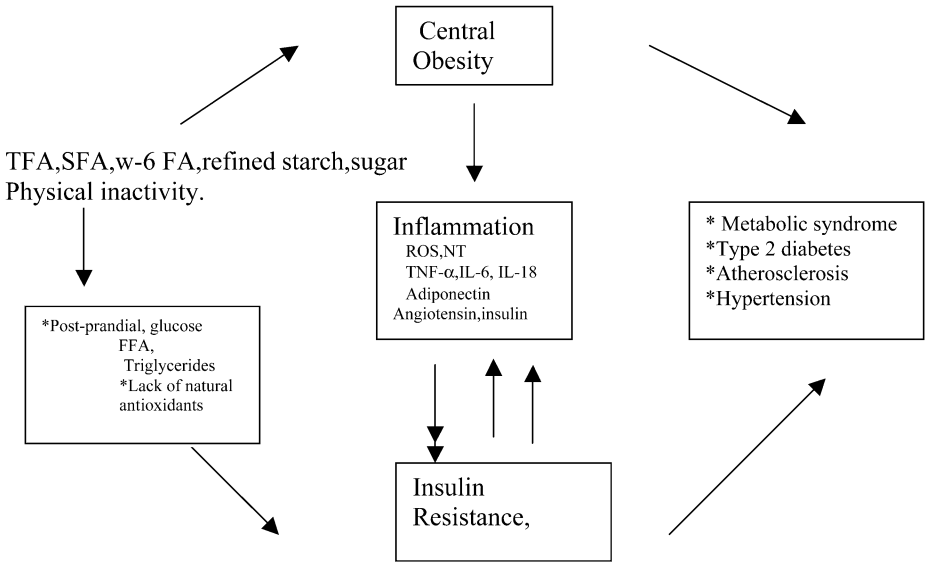


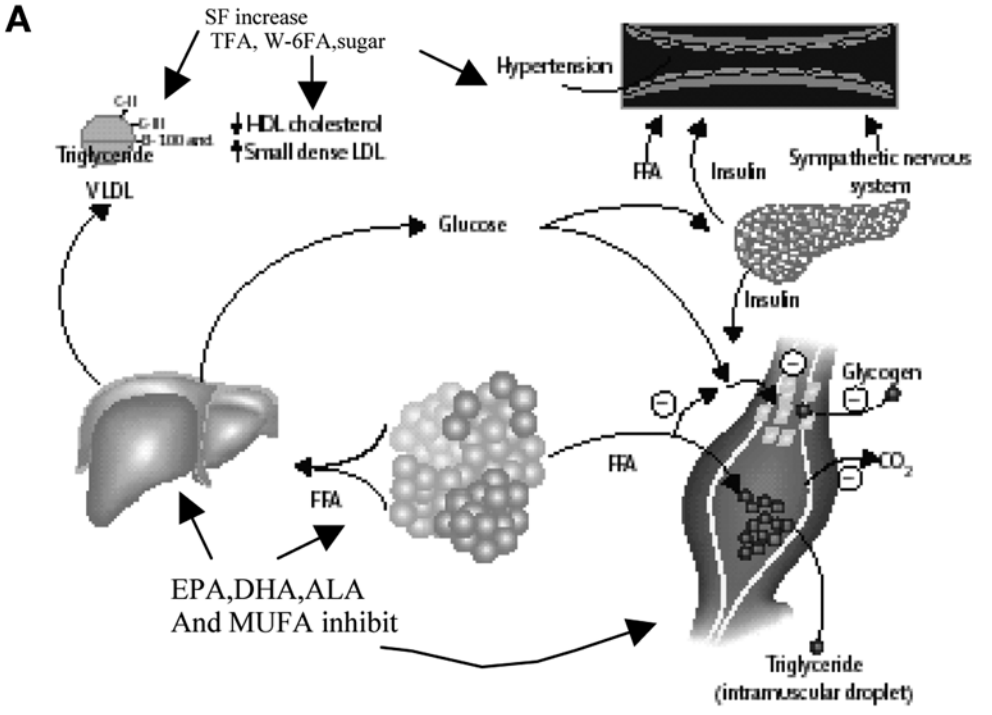
Fig. 1. Diet, inflammation and insulin resistance (modified from ref. *49*).

molecule-1 (ICAM-1), in association with raised plasma concentrations of IL-6 and TNF-α *(49)*. A high-fat meal *(63)* may increase the circulating levels of IL-18, a pro-inflammatory cytokine believed to be involved in plaque destabilization associated with the simultaneous decrease of circulating adiponectin, an adipocyte-derived protein with insulin sensitizing, anti-inflammatory, and antiatherogenic properties *(6,7,64)*.

9. DIET, INSULIN RESISTANCE, AND METABOLIC SYNDROME

Biological dysfunctions, found in diabetes, obesity, and the metabolic syndrome include, among others, increases in the circulating levels of metabolites (e.g., FFA, triglycerides, and cytokines such as TNFα and IL-6). Administration of a marcronutrient causes a shift towards oxidative stress and inflammation, which in turn may reduce insulin sensitivity (Fig. 1). FFA, as well as proinflammatory markers, have been shown to predict type 2 diabetes independent of known risk factor *(65,66)*. Both FFA and TNF-α have also been shown to activate inhibitor K kinase β (IKK-β) in adipocytes and hepatocytes, which can then increase the serine phosphorylation of insulin receptor substrate1 (IRS-1), with subsequent reduction in insulin-dependent tyrosine phosphorylation of IRS-1, and ultimately glucose transport *(67)*. IKK-β is a serine kinase that controls the activation of NK-κB, a transcription factor associated with inflammation. IRS-1 may be directly phosphorylated by IKK-β at serine residues, representing a novel class of substrates for IKK-β *(68)*. In one recent study *(69)*, in which hepatic expression of the lκBα super-repressor, that reduces IKK-β activity, reversed the phenotype of wild-type mice fed a high-fat diet.

It is possible that lipid accumulation in the liver leads to subacute hepatic "inflammation" through NK-κB activation and downstream cytokine production resulting in insulin resistance both locally in the liver as well as systemically. Circulation of IL-6 in plasma was high and was associated with insulin resistance in both men and in obese or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TFA, W-6FA,sugar
Hypertension
C-II
C-III
B-100 and
Triglyceride
HDL cholesterol
Small dense LDL
FFA
Insulin
Sympathetic nervous system
VLDL
Glucose
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Glycogen
CO_2
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EPA,DHA,ALA
And MUFA inhibit
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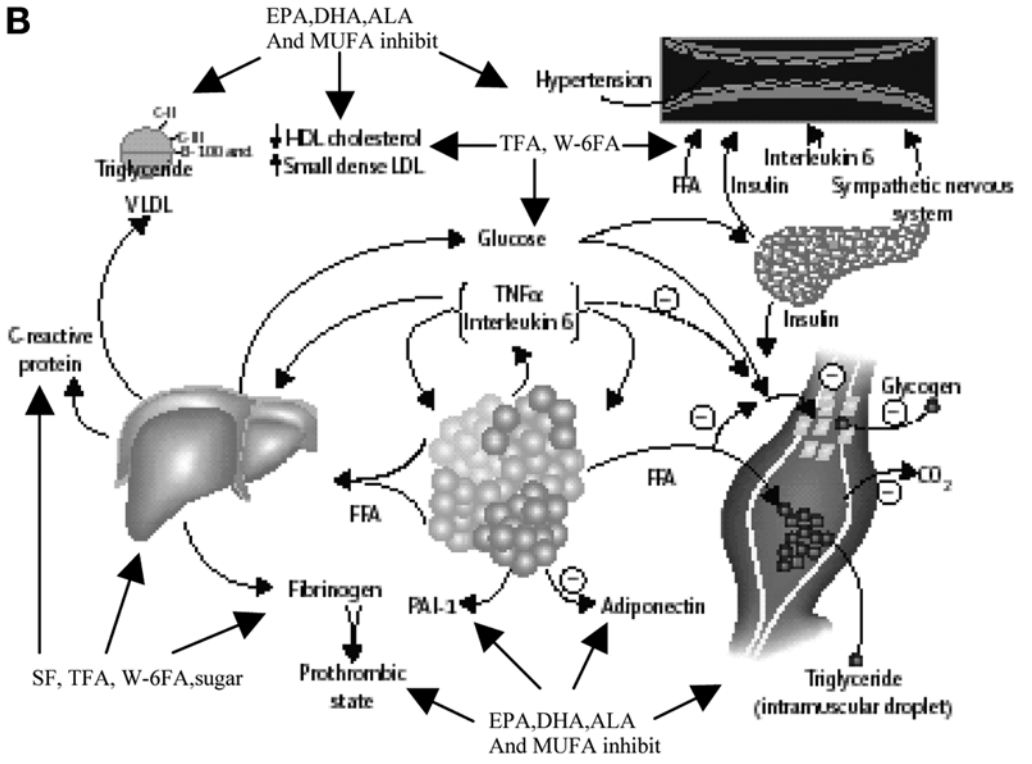
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FFA
Insulin
Sympathetic nervous system
VLDL
Glucose
TNFα
Interleukin 6
Insulin
C-reactive protein
Glycogen
CO_2
FFA
FFA
Fibrinogen
PAI-1
Adiponectin
SF, TFA, W-6FA,sugar
Prothrombic state
Triglyceride
(intramuscular droplet)
EPA,DHA,ALA
And MUFA inhibit

hyperandrogenic women *(49,50)*. Circulating IL-6 levels and insulin sensitivity relationships seem to occur in parallel with increases in plasma FFA. In contrast with IL-6 and TNF-α, adiponectin mRNA is reduced in adipose tissue from patients with type 2 diabetes *(64)*. It appears that low-adiponectin production contributes to insulin resistance; there is evidence that adiponectin decreases circulating FFA levels by increasing fatty acid oxidation by skeletal muscle *(70)*. The endogenous proinflammatory potential may be greater in the post-prandial phase resulting from an imbalance in pro- and anti-inflammatory cytokines, particularly following the ingestion of rapidly absorbed foods. Modification of circulating FFA levels may mediate this effect and blunting of the anti-inflammatory actions of insulin may also play a role. Insulin causes a suppression of NK-κB at physiologically relevant concentrations, thus reducing the production of some of its transcripts, namely IL-6 and TNF-α *(49)*. This benefit of insulin has been related to its ability to induce the release of nitric oxide and to enhance the expression of constitutive nitric oxide synthase (Fig. 2A and 2B).

10. CARDIOPROTECTIVE AND PROINFLAMMATORY DIETS

The vascular effects of high sugar and high fat meals have greatly increased our understanding about the role of diet on atherothrombosis *(49,50)*. There is an increased flux of nutrients in the post-prandial state which is associated with an increase in circulating levels of proinflammatory cytokines, recruitment of neutrophils, and oxidative stress. The generation of ROS may be a common ground for all these findings and may help in the understanding of current dietetic recommendations from the International College of Cardiology, emphasizing increased consumption of fruits, vegetables (400 g/d), nuts (50 g/d), grains (400 g/d), and spices. These foods are rich in natural antioxidants, phytochemicals, and fiber that help fight the oxidant wave of meals. Decreasing the

Fig. 2. *(Opposite page)* Pathophysiology of the metabolic syndrome (insulin resistance). **(A)** FFA are released in abundance from an expanded adipose tissue mass. In the liver, FFA produce an increased production of glucose, triglycerides, and secretion of VLDL. Associated lipid/lipoprotein abnormalities include reductions in HDL cholesterol and an increased density of LDL. FFA also reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. Associated defects include a reduction in glucose partitioning to glycogen and increased lipid accumulation in triglyceride (TG). Increases in circulating glucose and to some extent FFA increase pancreatic insulin secretion resulting in hyperinsulinemia. Hyperinsulinaemia may result in enhanced sodium reabsorption and increased sympathetic nervous system (SNS) activity and contribute to the hypertension as might increased levels of circulating FFA. **(B)** Superimposed and contributory to the insulin resistance produced by excessive FFA is the paracrine and endocrine effect of the proinflammatory state. Produced by a variety of cells in adipose tissue including adipocytes and monocyte-derived macrophages, the enhanced secretion of IL-6 and TNF-α among others results in more insulin resistance and lipolysis of adipose tissue triglyceride stores to circulating FFA. IL-6 and other cytokines also are increased in the circulation and may enhance hepatic glucose production, the production of VLDL by the liver and insulin resistance in muscle. Cytokines and FFA also increase the production of brinogen and plasminogen activator inhibitor-1 (PAI-1) by the liver that complements the overproduction of PAI-1 by adipose tissue. This results in a prothrombotic state. Reductions in the production of the anti-inflammatory and insulin sensitizing cytokine adiponectin are also associated with the metabolic syndrome and may contribute to the pathophysiology of the syndrome. PAI1, plasminogen activator inhibitor 1; FFA, free fatty acids. Reprinted with permission from ref. *1*.

intake of trans- and saturated fatty acids and increasing the consumption of ω-3 fatty acids (e.g., flax seed, mustard, and canola oil) and MUFAs (e.g., olive oil) are also considered important strategies to reduce CAD and metabolic syndrome *(1–3)*. There is evidence that these two strategies are also associated with a reduced inflammatory status. In the Nurse's Health Study, levels of C-reactive protein and markers of endothelial dysfunction were 73% higher in the highest quintile of TFA intake, compared with the lowest quintile *(71)* and low-cholesterol/low-saturated fat diets are associated with mitigation of low-grade systemic inflammation which correlated with reduction of plasma C-reactive protein levels *(72)*. Cross-sectional study from the Nurse's Health Study I cohort demonstrated lower concentrations of many markers of inflammation and endothelial activation, including C-reactive protein, IL-6, and E-selectin, among those in the highest quintile of ω-3 fatty acids, when compared with the lowest quintile *(73)*. Because a high intake of ω-6 fatty acids may reduce the known beneficial effects of ω-3 fatty acids on CAD risk *(11)*; a combination of both types of fatty acids in a ratio of 1:1 as advised by Paradigm Institute, which may be associated with the lowest level of inflammation *(74)*. However, it seems that ω-6:ω-3 ratio in the diet should be <5.0 to have optimal benefit of these fatty acids in the prevention of CVD, type 2 diabetes and metabolic syndrome.

Because free radical stress is supposed to play a key role in the development of atherosclerosis, antioxidant-vitamin supplementation has been suggested for the treatment and prevention of chronic diseases, including CAD *(5)*. The encouraging results of short-term trials in participants with coronary atherosclerosis were not confirmed in large-size intervention trials. It appears that it is wrong to focus on a single element of the diet and guidelines from some professional or governmental panels recommend consumption of vitamins and minerals from food sources rather than from supplements *(5,74)*. A shift toward energy dense, refined, ready prepared foods with a high glycemic index (e.g., refined starches and sugar) and unhealthy lipids (e.g., TFA, SF, ω-6 rich oils) and poor in phytochemicals and fiber have been adopted by increased number of people and populations in the Western world and in the urban populations of middle income countries in the last few decades *(49,50)*. These changes in diet can cause inactivation of innate immune system through excessive production of proinflammatory cytokines and reduced production of anti-inflammatory cytokines that may result in generation of inflammatory milieu, causing insulin resistance and endothelial dysfunction. These changes in diet, in conjunction with inadequate physical activity, appear to be responsible for the development of positive energy balance, weight gain, and central obesity, which is widely acknowledged as an endocrine organ secreting an increased number of mediators, including proinflammatory cytokines *(75)*. Central obesity is a key promoter of low-grade systemic inflammation *(76,77)* and is characterized by the most severe metabolic abnormalities *(49,50)*. It seems that subjects with abdominal adiposity are particularly prone to the proinflammatory effects of unhealthy diets. The changes in dietary patterns that have occurred in recent years are characterized by the intake of large amount of foods that are faster to prepare and produce health damage.

One prospective Coronary artery risk development in young adults (CARDIA) study indicated that frequent fast food intake caused weight gain and the risk of insulin resistance over a period of 15 yr *(78)*.

The Quebec Family Study showed that a decrease in the consumption of fat-foods or an increase in consumption of whole fruits resulted in a lower increase in body weight and adiposity indicators over a 6-yr follow-up *(79)*. However, no specific dietary recommendations have been advocated by health agencies for the treatment of insulin resistance or the metabolic syndrome *(5,47,48)*. Given that metabolic syndrome is an identifiable and potentially modifiable risk state for both type 2 diabetes and cardiovascular disease, adopting a healthy dietary pattern may reduce the potential risk of these diseases *(80–84)*.

In one study, Knoops et al. *(75)* demonstrated that in European men and women between the ages of 70 and 90, who adhered to a Mediterranean-style diet representing a solid example of a healthy dietary pattern (e.g., moderate alcohol consumption, nonsmoking status, and physical activity) was associated with a lower rate of all-cause mortality. This combination of healthy diet and lifestyle was associated with a mortality rate of about one-third that of those with none or only one of these protective factors. In another larger study, involving about 22.000 adults, showed an inverse correlation between adherence to a Mediterranean-style diet and death *(76)*. In particular, an approximate 2/9-fold increase in Mediterranean diet score was associated with a 25% reduction in total mortality and a 33% reduction in CHD mortality.

Two main intervention trials that adhered to the whole diet approach also supported this epidemiological evidence. In the Lyon Diet Heart Study *(82)*, 605 patients who had myocardial infarction were randomly assigned to a "Mediterranean-style" diet or a control diet resembling the American Heart Association Step I diet. The Mediterranean diet model supplied 30% of energy from fats and <10% of energy from saturated fatty acids, whereas the intake of 18:3 (n-3) (α-linolenic acid) provided >0.6% of energy. After a mean follow-up of 27 mo, the risk of new acute myocardial infarction and episodes of unstable angina was reduced by ~70% by the Mediterranean diet, Moreover, total mortality was also reduced by 70%. Singh et al. *(85)* tested an "Indo-Mediterranean diet" in 1000 patients in India who had existing coronary disease or were at high risk for developing coronary disease. When compared with the control diet, the intervention diet—characterized by increased intake of mustard or soyabean oil, nuts, vegetables, fruits, and whole grains—reduced the rate of fatal myocardial infarction by one-third and the rate of sudden death from cardiac causes by two-thirds.

Acute coronary syndromes (ACS) is associated with hyperglycemia, hypertriglyceridemia, hyperinsulinemia, increased FFA, free radical stress, IL-6, TNF-α which are also indicators of metabolic syndrome (Table 1). There is deficiency of antioxidant vitamins and ω-3 fatty acids, among these ACS patients which appear to be responsible for complications and deaths *(6,7,86–92)* (Tables 2–4). Recent studies *(49,50,85–90)* indicate that eating high-fat, refined, carbohydrate-rich fast foods (western diet) can produce a similar proinflammatory state in our body, resulting in endothelial dysfunction, which may have adverse effects in patients with ACS *(91)*. It is therefore logical to avoid western diet in patients with ACS and administer Indo-Mediterranean foods which may be beneficial to vascular endothelium and myocardium (Tables 2–5). There is no precise and proven guideline for dietary advice in patients with ACS, which may be protective against recurrent cardiac events. A Columbus soup (tomatoes, grapes, vegetables, walnuts, almonds + linseed and olive oil) or yogurt containing walnuts, almonds, raisins, could be prepared for ready use, for nonpharmacological intervention,

among patients of ACS. These foods appear to be protective against metabolic syndrome *(87–99)*. Such recipies have been commonly used by us in our studies and clinical practice *(87–91)* (Tables 2–4). Therefore for prevention of metabolic syndrome, eat 400 g/d of fruits and vegetable and another 400 g/d of legumes and whole grains, and 50 g/d of almonds and walnuts, in conjunction with 25–50 g/d of Columbus oil (olive oil 90 %+flax seed oil 10%). Increased physical activity to control sedentary behavior is important in urban and affluent populations for prevention of metabolic syndrome as part of Columbus lifestyle *(100)*. Apart from from designers foods and lifestyle changes, drug therapy with fibrates, statins, coenzyme Q10, ω-3 fatty acids, ACE-inhibitors, beta blockers, metformin and glitozones may be administered for prevention and treatment of metabolic syndrome (Tables 2, 5).

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20 Immune Modulation and Cancer Resistance

Enhancement by ω-3 Fatty Acids

Erin M. O'Connell, Patricia D. Schley, and Catherine J. Field

Abstract

The incidence of cancer of the colon and breast has been steadily increasing in Western countries. The etiology of colon and breast cancer is complex, involving both genetic and environmental factors, including diet. Epidemiological and experimental studies have linked a high dietary intake of ω-6 polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2, LA) to an increased risk of cancers of the breast and colon, especially in association with a low intake of ω-3 PUFAs such as docosahexaenoic acid (C22:6, DHA) or eicosapentaenoic acid (EPA) acid. Changes in anticancer immune defenses resulting from this dietary shift in PUFA balance may facilitate cancer progression. Altering the ω-6:ω-3 ratio has been demonstrated to influence immune function in other diseases, however less is known about their effect on immune surveillance during cancer. The biochemical mechanisms whereby decreases in the ω-6:ω-3 ratio of the diet (via increasing DHA and EPA intake) modulate immune function and inhibit tumor growth are not well established but mechanistic studies suggest that this may occur at several sites in the cell. In immune cells, this includes ω-3 PUFA competitively inhibiting arachidonic acid metabolism, altering membrane composition, modifying cell signaling processes and/or changing the expression of genes. In this chapter, we will review the role of the immune system in cancer prevention and promotion, with a focus on evidence from animal and human studies that implicates changes in the level of ω-6 and ω-3 fatty acids in the diet on immune protection from breast and colon cancer. Evidence that points to possible immune mechanisms will also be discussed. Although at the present time it is too early to make any clear recommendations regarding the precise ratio of ω-6:ω-3 PUFA in the diet to impact favorably on anti-cancer defenses, the available evidence presented in this chapter should encourage public health authorities to consider designing primary prevention campaigns to reduce the ω-6/ω-3 ratio of the Western diet by promoting increases in ω-3 PUFA consumption in the population.

Key Words: Colon cancer; breast cancer; natural killer cells; immune surveillance; inflammation; docosahexaenoic acid; eicosapentaenoic acid.

1. INTRODUCTION

Malignant neoplasia of the colon and breast are two of the most prevalent diagnoses in oncology in Western countries. The incidence of both of these forms of cancer in industrialized countries has increased since the early 1970s. Although not completely established, the etiology of colon and breast cancer is likely complex, involving both genetic and environmental factors. At least one-third of all human cancers may be

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associated with factors related to a Western diet and influenced by lifestyle and physical exercise *(1)*. Dietary fat is thought to be one of the main risk factors based on reports of positive correlations between dietary fat intake and increased risks for cancers of the breast, colon and prostate *(2–7)*. Although the role of individual fatty acids in human cancer risk has hitherto been poorly investigated, epidemiological and clear experimental data have now linked a high dietary intake of ω-6 polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2, LA), especially in association with a low intake of ω-3 PUFAs such as docosahexaenoic acid (C22:6, DHA), to increased risks for cancers of the breast and colon *(2,8–10)*. While there is limited evidence to suggest that ω-3 PUFA influence cancer cell initiation (i.e., the induction of irreversible genetic damage), numerous studies suggest that ω-3 PUFA can modulate tumor promotion (i.e., the selective outgrowth of initiated cells). In experimental animal models, ω-6 PUFAs have been demonstrated to enhance tumorigenesis and metastasis by several mechanisms, whereas ω-3 PUFAs inhibit the growth of many cancer cell types *(2)*. It is important to consider that the complete profile of fatty acids in the diet, and in particular the content of ω-6 fatty acids in the diet, plays a significant role in modulating the immune effects of ω-3 PUFAs. It is not as simple as studying the effect of absolute levels of ω-3 fatty acids; it is imperative to study their effects in relation to ω-6 levels, as the effects of a high ω-3 fatty acid concentration in the diet may be completely obliterated if the ω-6 content is equally high. It is for this reason that many studies investigate the ω-6:ω-3 ratio.

It is well established that feeding DHA and eicosapentaenoic acid (EPA, from 5 to 20% [w/w], as fish oil) reduces the growth of tumors in rodents, including tumors of the mammary gland *(9,11,12)*, colon *(9)*, prostate *(13)*, liver *(14)*, and pancreas *(15)*. Work in human cancer cell lines has convincingly demonstrated that the long chain polyunsaturated ω-3 fatty acids, DHA, and/or EPA can reduce the growth of many different human tumor types, including breast *(16,17)*, colon *(18)*, pancreatic *(19)*, chronic myelogenous leukemic *(20)*, and melanoma *(21)* cell lines. There are many studies suggesting effects of dietary ω-6/ω-3 balance on the initiation, growth and apoptosis of tumor cells *(22,23)*.

The immune system plays an important role in cancer defense (*see* Fig. 1). There is a progressive decrease in many immunosurveillance defences in animal models of cancer *(24)* and humans with cancer *(25)*. Changes in the ω-6:ω-3 ratio have been demonstrated to influence immune function in other diseases *(26–29)*, however less is known about their effect on immune surveillance in breast and colon cancer. In this chapter, we will review the role of the immune system in cancer prevention and promotion, with a focus on evidence from animal and human studies that implicates the effect of changing the level of ω-6 and ω-3 fatty acids in the diet on immune protection from breast and colon cancer.

2. ROLES OF THE IMMUNE SYSTEM IN CANCER PREVENTION

The mammalian immune system is an adaptive defence system composed of a complex dynamic network of cells and molecules that protect the host from challenges that originate from both the external (foreign pathogens) and internal (transformed, infected or damaged cells) environment. Immunity includes both innate (nonspecific)

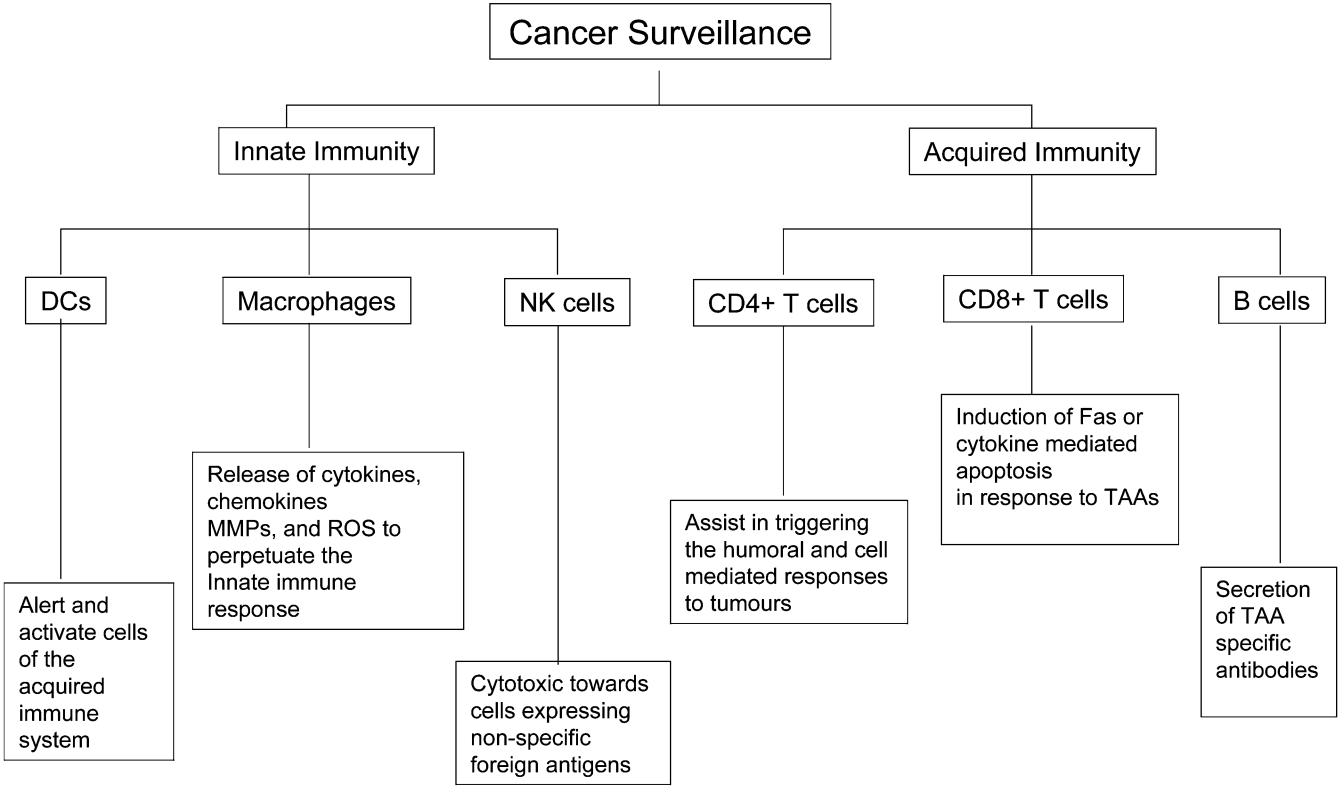

Fig. 1. The role of the immune system in cancer surveillance. Abbrs: NK, natural killer cells; DCs, dendritic cells; MMP, matrix metalloproteinases; ROS, reactive oxygen species; TAA, tumor associated antigens.

and acquired (specific or adaptive) components (*see* Fig. 1). Innate immunity represents a set of resistance mechanisms that are not specific to a particular pathogen or antigen. In contrast, the acquired component displays a high degree of specificity as well as the remarkable property of memory. As the initiation of the acquired immune response requires some time, innate immunity provides the first line of defence during the critical period just after the host's exposure to a pathogen/challenge. The innate and acquired immune systems work together to elicit an effective immune response. It is now well established that each stage of cancer development is exquisitely susceptible to regulation by immune cells *(30)*. Full activation of the cells in the acquired immune system that are directed at the tumor might result in eradication of malignant cells, whereas chronic activation of certain immune cells in or around pre-malignant tissue might actually promote tumor development *(30)*.

2.1. Innate Immune System and Cancer Protection

The unique characteristic of innate immune cells is their inherent ability to rapidly respond when tissue injury/invasion occurs, without memory of previous assaults or antigen specificity. Innate immune cells, such as dendritic cells (DCs), natural killer (NK) cells, macrophages, neutrophils, basophils, eosinophils and mast cells, are the first line of defence against foreign pathogens (*see* Fig. 1). Dendritic cells (DCs), macrophages, and mast cells serve as sentinel cells that are located in tissues and continuously monitor their microenvironment. When tissue homeostasis is perturbed, such as would occur with the growth of a transformed cell, sentinel macrophages and mast cells release soluble mediators, such as cytokines, chemokines, matrix remodeling proteases, and reactive oxygen species (ROS), and bioactive mediators such as histamine (only released by mast cells), that induce mobilization and infiltration of additional leukocytes into damaged tissue, a process that is known as inflammation. Acute activation of innate immunity sets the stage for activation of the acquired immune system. NK cells are lymphocytes of the innate immune system that exert their biological activity by a triad of functions: cytotoxicity, cytokine secretion and co-stimulation; these cells are critical to the elimination of tumor cells *(31)*. The major function of NK cells is the identification and elimination of cells that become malignant before they can become a tumor *(31)*. Once a tumor is eliminated, immune cells are crucially involved in normalizing cell-proliferation and cell-death pathways to enable re-epithelialization and new extracellular matrix synthesis. Because of their enormous plasticity, immune cells exert multiple effector functions that are continually fine-tuned as tissue microenvironments are altered.

2.2. Acquired Immune System and Cancer Protection

Immunosurveillance, the ability to detect and destroy tumor cells, is an important role of the cellular arm of the immune system (*see* Fig. 1) *(32)*. Induction of the acquired immune responses requires direct interactions with mature antigen-presenting cells within a proinflammatory milieu. The key players in this arm of the immune system are T- and B-lymphocytes. Antigen recognition by T- and B-cells is dependent on the expression of antigenic peptides bound to a major histocompatibility complex (MHC)-derived molecule. MHC molecules belong to two main classes, class I and class II, and have distinct structural and functional features and are involved in different types of

immune responses. Individual B- and T-cells are antigenically committed to a specific unique antigen, and clonal expansion upon recognition of foreign antigens is required to obtain sufficient antigen-specific B- and/or T-lymphocytes to counteract a challenge. Therefore, the kinetics of primary acquired responses are slower than innate responses. During the activation of the acquired immune system a subset of lymphocytes differentiate into long-lived memory cells, which will react with a larger response upon subsequent exposure to the same antigen. Colorectal *(33)* and breast *(34)* carcinomas have long been considered poorly immunogenic and substantially refractory to immunotherapy. However, during the last decade, considerable progress has been made in both the molecular characterization of T-cell defined tumor associated antigens (TAA) and in the methods allowing detection of antigen-specific T-cell responses and these have modified the scientific community's perspective on this issue. It is now generally accepted that tumor cells in the colon and breast can express proteins able to activate the immune system and to become targets for a T-cell-mediated response *(33,34)*.

T-cells are the main effector arm of acquired and antigen-specific cellular immune responses, and play a central role in immunity against cancer *(33)*. The specific T-cells that likely play the key role in the anti-tumor immune responses are cytotoxic T-lymphocytes (CTLs), classically $CD8^+$ T-cells *(35)*. Cytotoxicity by these cells is exerted directly through the Fas or perforin pathway and/or indirectly by the release of cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ *(35)*. The released TNF-α binds to its receptor on the transformed cell and triggers a caspase cascade, leading to target-cell apoptosis *(35)*. The released IFN-γ induces transcriptional activation of the MHC class I antigen presentation pathway and Fas in target cells, leading to enhanced presentation of endogenous peptides by MHC class I, and increased Fas-mediated target-cell lysis *(35)*. The cytolytic activity of CTL requires direct cell–cell contact, which can result in apoptosis of tumor cells, via either the release of lytic proteins or secretory lysosomes *(35)*. Unfortunately, human tumors frequently lose or down-regulate expression of class I MHC molecules *(33)* and this abrogates antigen recognition and tumor cell killing by $CD8^+$ CTLs *(33)* which would enable tumor cells to escape T-cell control. There is also evidence that some tumor cells can up regulate their expression of Fas-ligand (normally expressed on T-cells) thereby evading Fas-mediated apoptosis. In addition to T-cell-mediated responses, several studies have also revealed in the serum of colorectal cancer patients the presence of TAA-specific antibodies of different immunoglobulin (Ig) classes, suggesting a role for B cells in cancer surveillance *(33)*. $CD4^+$ cells, through their production of cytokines, may assist in triggering the humoral and the cell-mediated immune responses to tumors *(32)*.

2.3. Role of the Immune System in Cancer Promotion

Many factors influence tumor induction and growth in colon cancer, including a range of cytokines and growth factors *(8)*. Experimental data has demonstrated in breast cancer that infiltrating immune/inflammatory cells or tumor associated macrophages (TAM) and tumor associated dendritic cells (TADC) secrete a diverse repertoire of growth factors and proteases that enhance tumor growth by stimulating angiogenesis *(34,36)*. These factors include tumor growth factor (TGF)-β, which promotes tissue remodeling via secretion of matrix metalloproteinases (MMPs) *(33)*. Indeed, experimental data in mouse models provide clear demonstration that macrophage recruitment

at the tumor site may play an active role in promoting tumor progression and metastasis *(30,33,36)*. Additionally, TAA have been identified in breast cancer, some of which appear to play a critical role in tumorigenesis *(34)*. It has also been suggested that chronically activated TAM and TADC indirectly contribute to cancer development through suppression of antitumor adaptive immune responses. Perhaps the most compelling clinical evidence for a causative link between chronic inflammation and cancer development comes from epidemiological studies reporting that inhibiting chronic inflammation in patients (using anti-inflammatory drugs in persons with pre-malignant disease or who are predisposed to cancer development) has chemopreventative potential *(36)*.

Whereas it has become generally accepted that chronic activation of innate immune cells contributes to cancer development, the role of adaptive immune cells in promoting cancer is still a matter of debate. It is hypothesized that chronic inflammation might activate T-cells to produce excessive amounts of cytokines, chemokines, MMPs, ROS, histamine, and other bioactive mediators that facilitate tumor initiation and growth, and disable the tumor-killing $CD8^+$ CTL response *(30)*. Potential tumor-promoting roles for B-lymphocytes and/or antibodies have also been proposed *(30)*.

2.4. Summary

Clinical and experimental studies now indicate that innate and adaptive immune cells are significant, albeit sometimes paradoxical, determinants of epithelial tumorigenesis. Breast cancers arise in immunocompetent hosts, and outmanoeuvre immune recognition and ultimately progress to widely disseminated disease *(37)*. In recent years, several explanations have been proposed to account for the inability of the immune system to recognize and reject antigenic breast tumors. There is increasing evidence pointing to the development of tolerance towards TAA to explain the failure of the immune system to reject breast tumors *(37)*. The balance between angiogenesis-promoting, -inhibiting, and -modulating influences by immune cells at given times and locations in the tumor microenvironment clearly has a potential to determine the overall course of tumor development *(36)*.

3. THE EFFECT OF ω-3 FATTY ACIDS AND ω-6:ω-3 RATIO ON THE IMMUNE SYSTEM IN ANIMAL MODELS OF BREAST AND COLON CANCER

The rodent studies examining the effect of changes in the ω-6:ω-3 ratio in the diet on breast and colon cancer are reviewed in Table 1. It is well established that long chain ω-3 PUFA can upregulate NK cell cytotoxicity as well as humoral and T-cell responses *(38)* that play a role in anticancer defense. Research from our laboratory has demonstrated that the influence of dietary ω-3 PUFA on the immune response differs between healthy rats and rats bearing the R3230AC mammary tumor *(11,12)*. Additionally, the complete profile of fatty acids in the diet, particularly the content of ω-6 fatty acids, influences the immune effect that results after feeding ω-3 fatty acids. Tumor-bearing rats fed long-chain ω-3 PUFA (50 g/kg of total fat) as part of a low PUFA diet had significantly increased NK cell cytotoxicity, splenocyte nitric oxide, IL-2, IFN-γ, and TNF-α production, and proportion of activated ($CD25^+$) $CD8^+$ and $CD28^+$ cells,

Table 1
Studies of Dietary ω-3 Fatty Acids and Tumor Growth in Animals

Reference	*Animal model*	*Tumor*	*Level of fat*	*ω-6:ω-3 ratio*	*ω-3 PUFA source*	*Length of feeding*	*Outcomes*
Hansen-Petrik et al. *(42)*	C57BL/6J $APC^{Min/+}$ mice	Spontaneous small intestinal adenoma	10% *(w/w)*	OA:7.6 AA:10.2 EPA:2 AA/EPA:3	EPA ethyl ester	8 wk	EPA ↓ tumor number by 68, 54, and 50% vs OA, AA, and EPA/AA. EPA feeding ↓ PGE_2 and 6-keto-PGF1a, EPA/AA eliminated any reductions seen in EPA alone.
Mukutmoni-Norris et al. *(39)*	BALB/c AnN mice	Mammary tumor 4526	20% *(w/w)*	FO:0.31 FS:1.9 SO:20	Fish Oil	Feeding time varied as mice were euthenized according to tumor volume	FO and FS significantly ↓ blood vascular area, mast cell number and macrophage infiltration into tumors compared with SO. SO ↑ VEGF two-fold vs FO.
Rao et al. *(45)*	F344 rats	AOM induced colon carcinoma	5% *(w/w)* in LFCO and 20% *(w/w)* HFFO and HFML	LFCO:7.8 HFFO:0.24 HFML:10	Fish Oil	8, 23, and 38 wk	HFML fed rats had significantly ↑ number of total ACF/colon compared with LFCO and HFFO at 8 and 23 wk. At 23 and 38 wk HFML showed significantly ↑ colon tumor incidence and multiplicity. HFML had a significantly ↓ apoptotic index compared with the other two groups. HFML significantly ↑ eicosanoid levels in colon tumors compared with HFFO and LFCO.

(Continued)

Table 1 *(Continued)*

Reference	*Animal model*	*Tumor*	*Level of fat*	*ω-6:ω-3 ratio*	*ω-3 PUFA source*	*Length of feeding*	*Outcomes*
Robinson et al. *(12)*	F344 rats	R3230AC mammary tumor	20% *(w/w)*	Low P/S diet:3.8 High P/S diet:7.7	Fish Oil	38	ω-3 feeding did not decrease tumor weight. The low P/S diet ↑ NK cell cytotoxicity, spleen NO and IL-2 production and activated $CD8^+$ and $CD28^+$ cells.
Robinson et al. *(11)*	F344 rats	R3230AC mammary tumor	20% *(w/w)*	Low P/S diet:3.8	Fish Oil	38	ω-3 feeding ↓ tumor weight by 31%. ω-3 PUFA ↑ T-cell activation, IFN-γ and TNF-α production.

Abbrs: APC, adenomatous polyposis coli; PGE_2, prostaglandin E_2; 6-keto-PGF1a, 6-keto-prostglandin F1a; OA, oleic acid; AA, arachadonic acid; EPA, eicosapentaenoic acid; FO, fish oil; FS, fish oil/safflower oil; SO, safflower oil; VEGF, vascular endothelial growth factor; AOM, azoxymethane; LFCO, low fat corn oil; HFFO, high fat fish oil; HFML, high fat mixed lipids; ACF, aberrant crypt foci; P/S, polyunsaturated/saturated; NK, natural killer; NO, nitric oxide; IL-2, interleukin-2; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.

whereas these immune enhancements were not found when ω-3 PUFA were supplemented in a high PUFA diet *(11,12)*. In addition, R3230AC tumor growth was reduced by 31% ($p = 0.1$) only in rats fed the ω-3 PUFA as part of the low PUFA diet *(11,12)*. It is possible that this reduced tumor growth results not only from the direct effects of the ω-3 fatty acids on the tumor itself but also to their ability to modulate the immune system and hence tumor cell recognition and killing.

Feeding a fish oil enriched or a mixed lipids (both fish oil and safflower oil) enriched diet with ω-3:ω-6 ratios of 3.2 and 0.52, respectively, decreased mast cell number and macrophage infiltration in solid mammary tumors in female BALB/cAnN mice compared with a safflower-only enriched diet with a ω-3:ω-6 ratio of 0.05 *(39)*. Given the potential for infiltrating innate immune cells such as macrophages to promote angiogenesis and tumor growth, this study supports a mechanism of reduced mammary tumor growth involving a reduction in inflammation. Further evidence has shown that ω-3 PUFA enriched diets decrease the number of microvessels in tumors derived from mouse or human mammary cell lines transplanted in mice *(39,40)*. Direct effects of ω-3 on angiogenesis have been studied and the modulation of eicosanoid production appears to be one mechanism underlying the anti-angiogenic potential of ω-3 PUFAs in colon cancer. Calviello et al. *(41)* recently found that ω-3 PUFAs regulate vascular endothelial growth factor (VEGF) expression both in vitro and in vivo. Both EPA and DHA reduced VEGF and cyclooxygenase (COX)-2 expression, as well as prostaglandin E_2 (PGE_2) levels in HT-29 cells. Furthermore, EPA and DHA inhibited extracellular signal-regulated kinase (ERK)-1 and -2 phosphorylation and hypoxia-inducible factor 1-α (HIF1-α), downstream regulators of the PGE_2 signaling pathway. Both PUFA also decreased the growth of tumors in nude mice as well as microvessel formation, VEGF, COX-2, and PGE_2 in tumors. These results are supported by the study by Mukutmoni-Norris et al. *(39)* demonstrating that feeding a 20% *(w/w)* fish oil and mixed safflower oil/fish oil diets reduced blood vascular area and VEGF levels compared with safflower oil alone in mice with 4526 mammary tumors.

In the *APC*$^{min/+}$ model of multiple intestinal neoplasia, mice fed a diet supplemented with EPA had 68% fewer tumors compared with control animals fed an oleic acid rich diet, whereas arachidonic acid (AA) feeding had no effect when compared with the oleic acid-fed animals. The reduced tumor growth associated with EPA feeding was consistent with significant reductions in intestinal AA and associated prostaglandins (PGs) *(42)*. Feeding a diet of combined EPA and AA at a 1:1 ratio abolished the antitumorigenic effect of EPA, further indicating that the ratio of ω-6:ω-3 in the diet is important to cancer promotion *(42)*.

The azoxymethane (AOM) rat preclinical model has been shown to be a particularly good model for assessing the effects of ω-3 PUFAs on the development of colon cancer. In this model, the colons of F344 AOM-treated rats have similar morphological and histochemical properties to those of humans, and the biological behaviour of AOM-induced rat colon carcinomas is similar to that of human colon carcinomas *(43,44)*. A study conducted by Rao et al. *(45)* using this particular model compared the effects of high fat diets containing mixed lipids rich in saturated fatty acids (HFML) with an ω-6:ω-3 ratio of 10:1 or fish oil (HFFO) with a ω-6:ω-3 of 0.24:1 on the different stages of colon carcinogenesis. The HFML diet significantly increased colon tumor incidence and multiplicity when compared with the HFFO diet.

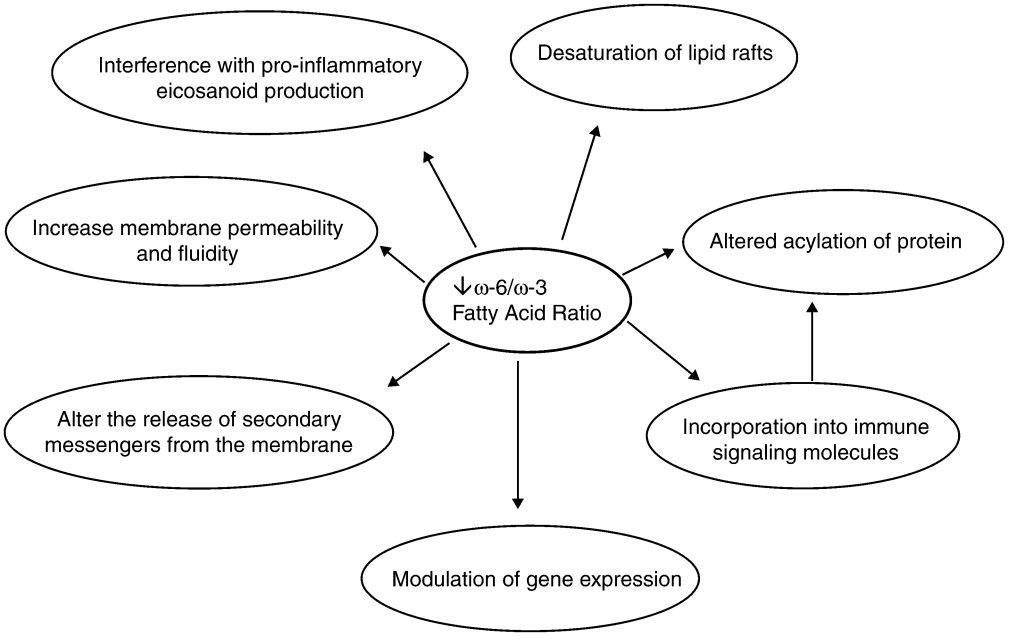

Fig. 2. Postulated mechanisms for ω-3 fatty acid modulation of immune function and cancer.

Furthermore, the animals fed the HFML diet produced significantly higher levels of eicosanoids compared with the HFFO animals. This study reinforces previous animal and epidemiological studies that both the type and amount of fatty acids in the diet play critical roles in carcinogenesis.

It is important to remember that while the animal studies are quite promising, very high levels of ω-3 PUFAs (often 20–24% by weight) are fed in these diets *(46)*. Although animal results may be proof of concept, it is essential to determine in human studies what level of ω-3 PUFA can be tolerated or implemented in the human diet and whether this amount could effectively immunomodulate and suppress cancer growth.

4. MECHANISMS TO EXPLAIN THE EFFECTS OF ω-3 PUFA ON IMMUNE AND TUMOR CELLS

The biochemical mechanisms whereby ω-3 fatty acids modulate immune function and inhibit carcinogenesis are not well established. This section will focus on postulated mechanisms by which ω-3 PUFA influence immune function and tumor growth (*see* Fig. 2).

4.1. ω-3 PUFA Interfere With Eicosanoid Production From AA

ω-6 and ω-3 fatty acids are incorporated into the phospholipids of cell membranes of many cell types, including immune and tumor cells, following ω-6 or ω-3 PUFA inclusion in the diet or cell culture media *(12,47–51)*. LA (C18:2ω-6) and linolenic acid (LNA, C18:3ω-3) can be metabolized to AA (C20:4ω-6, AA) or EPA (C20:5ω-3),

respectively, by a system of elongase and desaturase enzymes. AA and EPA from membrane phospholipids are substrates for the biosynthesis of various eicosanoids, namely prostaglandins (PGs) and thromboxanes (TXs) via COX enzymes, and leukotrienes (LTs) via lipoxygenase (LOX) enzymes.

ω-3 fatty acids interfere with eicosanoid production through multiple mechanisms. ω-3 fatty acids decrease the supply of AA for conversion to eicosanoids by displacing AA from membrane phospholipids. Increasing the ω-3 PUFA content of the diet or culture media has been shown to produce corresponding increases in the long chain ω-3 PUFA content of cell membranes at the expense of ω-6 PUFA, particularly AA *(52)*, thus reducing the amount of AA in phospholipids available for eicosanoid production. ω-3 PUFA also compete with ω-6 PUFA for desaturases and elongases, and because ω-3 PUFA have greater affinities for these enzymes than do ω-6 PUFA, ω-3 PUFA can reduce the elongation and desaturation of LA to AA *(53)*. In addition, EPA can compete with AA for conversion to eicosanoids by the COX and LOX enzymes. Higher levels of ω-3 PUFA in cell membranes will reduce the production of proinflammatory eicosanoids (i.e., PGE_2, LTB_4, TXA_2) from ω-6 PUFA, and increase the production of eicosanoids from ω-3 PUFA (PGE_3, LTB_5). The eicosanoids produced from ω-3 fatty acids have a lower biological potency than those produced from ω-6 fatty acids *(54,55)*. There is also some evidence that ω-3 fatty acids may actually decrease the expression or activity of COX enzymes involved in eicosanoid production *(10,56,57)*.

Increased PGE_2 and TXA_2 concentrations have been reported in breast cancer, and long term nonsteroidal anti-inflammatory drug (NSAID) usage has been associated with a reduced risk of breast cancer *(58,59)* supporting a role for eicosanoid reduction in the prevention of cancer. It appears that breast tumors vary in their expression of COX-2. Half et al. *(60)* report expression in 43% of invasive breast cancers, 63% of ductal carcinoma *in situ*, and in 80% of normal appearing breast tissue in close proximity to cancer. A recent study by Harris et al. *(61)* found that selective COX-2 inhibitors reduced breast cancer risk by 71%, indicating a role for inhibitors of the COX-2 enzyme as a chemopreventative cancer drug. COX-2 products, namely PGs, are reported to be elevated in malignant human breast cancer tissue *(62–64)* as well as in the plasma of breast cancer patients *(65)*. In addition, elevated mammary tumor levels of PGs have been associated with a poor prognostic outcome *(66)*. PGs, particularly PGE_2, have been demonstrated to increase angiogenesis in breast cancer *(40)* and to inhibit growth arrest and decrease apoptosis in the MDA-MB-231 cell line (a highly malignant breast cancer cell type) and in mice implanted with this tumor *(67)*.

Substantial evidence also exists to support the role of eicosanoids and COX-2 in the development of colon cancer. COX-2 overexpression in human cancer was first reported in 1994 by Eberhart et al. *(68)* who showed that COX-2 upregulation was present in colon adenocarcinoma but not in the accompanying normal mucosa. COX-2 has also been found to be overexpressed in animal models of colon cancer *(69)*. In a murine model of familial adenomatous polyposis (FAP), Oshima et al. *(70)* demonstrated that COX-2 null mice compared with mice expressing wildtype COX-2 had significantly fewer and smaller polyps. Additionally, treatment of the wild-type mice with a COX-2 inhibitor was effective in reducing polyp number. PGE_2 is the most abundant prostaglandin associated with colon cancer and as with breast cancer has been shown to

stimulate angiogenesis, increase cellular proliferation, and inhibit apoptosis *(71)*. Again, similar to breast cancer, increased PGE_2 expression is associated with a higher degree of tumor invasiveness *(71)*.

Numerous studies have demonstrated that changing the dietary balance of ω-3 and ω-6 fatty acids can alter eicosanoid production in vivo *(51,72–75)*. This is not unexpected given the fact that ω-3 and ω-6 PUFA are metabolized by the same enzyme system, and can displace each other in cell membranes. However, there are some conflicting studies regarding whether decreased eicosanoid production resulting from fish oil feeding has a detrimental effect on breast tumor growth *(9)*. Animal studies have shown significant decreases in eicosanoid (PGE_2) production with fish oil feeding without any detrimental effects on tumor growth *(76,77)*, suggesting that tumor growth, in these cases, was not affected by decreased PGE_2 levels. However, a greater number of studies discussed in detail in the previous section (*see* Section 3.3.), point to a significant role for EPA and DHA in the modulation of PG production and decreased tumorigenicity of breast and colon cancers *(39,41,42,45,60)*. The discrepancies among studies may result from the amount or proportion of ω-6 PUFA fed, or to the extent to which the tumor models used were modulated by inflammation.

4.2. ω-3 PUFA Alter Physical Properties of the Cell Membrane

ω-3 fatty acids alter physical properties of cell membranes, when incorporated into the membrane, as a result of their structure. EPA and/or DHA were shown to increase the membrane fluidity of erythrocytes in healthy subjects consuming fish oil *(78)* and of aortic endothelial cells in culture *(79)*, and to increase the permeability of tumor cells ex vivo *(80,81)*. Such changes may be functionally relevant to membrane-associated signal transduction in both tumor and immune cells, perhaps by affecting the binding of important ligands (e.g., cytokines, growth factors) to their receptors *(82,83)*, or by affecting the function of signaling molecules in proximity to the membrane.

4.3. ω-3 PUFA Alter the Release of Second Messengers From the Membrane

There is some evidence to suggest that ω-3 fatty acids may alter the formation of phospholipid-derived second messengers such as diacylglycerol (DAG) and ceramide. DAG is derived from the hydrolysis of phosphatidylinositol-4, 5-bisphosphate (PIP_2) by phospholipase C (PLC) enzymes *(84)*. DAG is the principle activator of several isoforms of protein kinase C (PKC), which is involved in a diverse array of cellular responses including proliferation *(85)*. The sphingolipid ceramide is derived from the hydrolysis of sphingomyelin by sphingomyelinase (SMase) enzymes. Ceramide is believed to mediate antiproliferative responses such as growth inhibition/cell-cycle arrest, apoptosis, differentiation, and senescence in response to certain cytokines or stress-causing agonists *(86)*. Ceramide modulates components of various signaling pathways (e.g., Akt, phospholipase D, PKC, and mitogen-activated protein kinases [MAPKs]) by regulating ceramide-activated protein phosphatases, kinases, and proteases *(86)*. Several studies have reported a decrease in DAG and ceramide production by splenocytes from mice fed EPA and/or DHA *(87–89)*. Conversely, ceramide levels were increased in Jurkat leukaemic cells exposed to DHA in vitro *(90)*. These opposing findings may be related to differences in ceramide metabolism in normal versus transformed cells (levels of ceramide appear to be particularly lower in tumor cells; *[86]*), but clearly

further research is needed to elucidate the effects of ω-3 fatty acids on this interesting area of signal transduction in immune cells.

4.4. ω-3 PUFA are Incorporated Into Immune Signaling Molecules

All phospholipids and some of their second messengers such as DAG and ceramide contain fatty acyl chains. Therefore, it is possible that changing the fatty acid composition of these molecules may alter their function *(91)*. In support, it has been demonstrated that EPA and DHA are incorporated into signaling molecules such as DAG with the supplementation of ω-3 PUFA in the diet *(87,92)*, and there is evidence to suggest that ω-3 PUFA-enriched DAG is less potent in activating PKC than ω-6 PUFA-enriched DAG *(93)*.

Many signaling molecules, including some tyrosine kinases, are reversibly acylated during signaling, which targets them to the cell membrane where they interact with other signaling molecules. It has been suggested that changing the fatty acid content of the diet (or the culture medium) may alter the acylation patterns of different signaling molecules *(94–96)*, affecting their ability to interact with the membrane. Alternatively, it is possible that changes in membrane fatty acid composition induced by changes in diet could alter the physical nature of the membrane regions to which acylated signaling molecules bind *(95)*, as described in the next section. Changes in early signal transduction events such as tyrosine kinase activation would modify downstream signaling events and is consistent with some of the reported changes in signaling molecules (*see* Section 4.3)when ω-3 PUFA are fed.

4.5. ω-3 PUFA Alter the Lipid and Protein Composition of Lipid Rafts

It has been more recently proposed that biological membranes are composed of membrane microdomains that segregate together as lipid "rafts." Lipid rafts are operationally defined as cholesterol-dependent membrane microdomains resistant to solubilization by nonionic detergents at low temperatures *(97)*. Lipid rafts are rich in sphingolipids and cholesterol (compared with the plasma membrane), whereas phospholipids are relatively depleted and contain predominantly saturated fatty acid residues *(98,99)*. Lipid rafts appear to be present in nearly all mammalian cell types *(97)*. A subset of specialized rafts termed caveolae have also been described, which are flask-shaped structures in the membrane that are enriched in the protein caveolin-1, and are believed to have several functions including endocytosis, cholesterol transport and signal transduction *(100)*.

Lipid rafts can include or exclude proteins to variable extents, leading to the hypothesis that rafts play a key role in signal transduction, perhaps functioning as platforms to concentrate signaling proteins *(101)*. The most extensive work relating to the function of rafts in signal transduction has come from the field of immunology, where there is strong evidence that lipid raft integrity is required for optimal T-cell receptor signal transduction and immune response *(101)*. Signaling through B-cell, epidermal growth factor (EGF), insulin, and integrin receptors is also thought to involve rafts *(101)*.

Relatively little is known about the effects of ω-3 PUFA incorporation into lipid rafts. Stulnig et al. demonstrated that PUFA treatment (both LA and EPA) of Jurkat T-cells in vitro led to a displacement of the Src family kinases Lck and Fyn from lipid rafts, compared with treatment with saturated or monounsaturated fatty acids *(102)*. Subsequently,

Stulnig et al. showed that EPA was significantly incorporated into lipid rafts (albeit to a lesser extent than into whole membrane phospholipids) following EPA treatment of Jurkat T-cells in vitro *(103)*. This was associated with a displacement of LAT (linker for activation of T-cells) from T-cell rafts *(103),* and a subsequent decrease in LAT-regulated phosphorylation of PLCγ *(104)*. Similarly, Diaz et al. *(105)* reported that DHA treatment of human peripheral blood mononuclear cells in vitro resulted in a displacement of phospholipase D (PLD) from lipid rafts, concomitant with an increase in PLD activity. The latter two studies are significant as they demonstrate that exclusion of certain signaling proteins from lipid rafts affects their function. Such changes in protein activity, as a result of either raft inclusion or exclusion, may be caused by conformational changes in these proteins when they are exposed to a different membrane environment, or they may be caused by interactions with other proteins within or outside of rafts *(105)*.

Two studies have examined PUFA effects on rafts in vivo. Fan et al. *(106)* demonstrated that EPA and DHA were selectively incorporated into the inner cytoplasmic leaflet phospholipids (i.e., PS and PE) of rafts and decreased raft sphingomyelin content in T-cells of fish oil-fed mice. This was the first study to demonstrate an effect of dietary ω-3 PUFA on raft lipid composition, and in particular, on the distribution of individual phospholipid classes in rafts. It has since been shown that dietary EPA and DHA are incorporated into caveolae from colonocytes of fish oil-fed mice *(107)*. Enrichment of ω-3 PUFA into caveolae was associated with a reduction in caveolae cholesterol, as well as a displacement of H-ras from caveolae, accompanied by a suppression of H-ras activity *(107)*.

It is not known how PUFA regulate raft localization of proteins, but it has been proposed that the substitution of unsaturated fatty acid residues for saturated residues in rafts may interfere with the interaction between acylated (e.g., palmitoylated, myristoylated) proteins and nonpolar lipids *(103)*. Alternatively, PUFA may alter the acylation of signaling proteins themselves, impacting on the ability of these proteins to localize within rafts. This idea is supported by a study by Webb et al. *(96)* who showed that both AA and EPA dramatically decreased Fyn acylation by palmitate, and subsequent raft localization, in COS-1 cells exposed to AA and EPA in vitro. The authors speculated that palmitate may have been replaced by AA and EPA. Regardless, the evidence presented above suggests that ω-3 PUFA modification of raft lipid and protein composition, leading to alterations in signal transduction, is a plausible mechanism to describe the anticancer and immunomodulating effects of ω-3 PUFA.

4.6. ω-3 PUFA Modulate Gene Expression

There is considerable evidence to indicate that ω-3 PUFA are capable of inducing changes in gene expression in a number of different cell types, including tumor and immune cells. The list of genes whose expression appears to be affected by fish oil or purified ω-3 fatty acids continues to grow and excellent reviews have been published *(54,108–110)*. As examples, ω-3 PUFA treatment has been associated with: reduced expression of prointerleukin-1β gene transcription by LPS-stimulated spleen cells from fish oil-fed mice *(111)*; suppressed IL-2 receptor alpha mRNA levels in splenic lymphocytes of mice fed EPA and DHA *(112)*; suppressed type IV collagenase mRNA expression in EPA-treated MDA-MB-435 breast cancer cells *(113)*; and reduced $p21^{ras}$ and COX-2 mRNA expression in mammary glands of rats fed fish oil *(56)*.

The precise mechanisms by which ω-3 fatty acids alter gene transcription is not known, but there is considerable speculation and new evidence that this might involve a class of nuclear receptors called peroxisome proliferator-activated receptors (PPARs). PPARs are ligand-activated transcription factors present in a variety of cell types, with diverse actions, mainly in lipid metabolism *(38,114)*. The PPAR family has at least three closely related members—PPARα (expressed in liver, kidney, heart and muscle), PPARγ (expressed in fat cells, large intestine, monocytic cells, and recently identified in a number of breast cancer cell lines), and PPARβ/δ (expressed in nearly all tissue) *(115)*. PPARs are fatty acid and eicosanoid receptors *(115)*. Various fatty acids have been shown to bind and activate the PPARs *(116–118)*; they can also be activated by certain COX and LOX metabolites of PUFA *(115)*. Recently, it was demonstrated that long-chain PUFA, including EPA, can bind to and activate PPARs *(118,119)*, providing a mechanism by which ω-3 PUFA could directly regulate gene expression in inflammatory cells.

ω-3 PUFA also have been shown to modulate the activity of the transcription factor nuclear factor (NF)-κB in monocytes and macrophages. NF-κB is a ubiquitously expressed transcription factor that regulates cytokine gene expression, cellular adhesion, cell survival, and programmed cell death *(120)*. The active transcription factor generally consists of a homo- or heterodimer of NF-κB family member subunits (e.g., p65, p50, p105, p100). In resting cells, NF-κB is sequestered in the cytoplasm through association with its inhibitor, I-κB (inhibitor of κB). Upon cell stimulation with cytokines (e.g., IL-1, TNF), growth factors (e.g., EGF, PDGF, insulin), or various stressors (e.g., ultraviolet radiation, pH, hypoxia, ROS), NF-κB dissociates from its inhibitor, and translocates to the nucleus where it transcribes genes *(121)*. Genes induced upon NF-κB activation include IL-2, IL-12, IFN-γ, COX-2, and inducible nitric oxide synthase *(122)*. Several studies provide convincing evidence that ω-3 PUFA significantly reduce NF-κB activation in monocytes and macrophages *(123–127)*. These studies suggest that ω-3 fatty acids have the potential to reduce chronic inflammation in the microenvironment of tumors by way of modulation of NF-κB activity. It is not clear how ω-3 PUFA modulate NF-κB activity, however, it has been suggested that these fatty acids modulate the activity of upstream signaling molecules involved in the activation of NF-κB *(123,125–127)*.

5. THE EFFECTS OF ω-3 FATTY ACIDS ON IMMUNE FUNCTION IN CANCER PATIENTS

Whereas no human clinical trials have been published that have specifically aimed to determine the effects of ω-3 PUFA on immune function with respect to cancer prevention, a number of clinical human studies have examined the effects of ω-3 PUFA on immune changes during cachexia. Cachexia is a wasting syndrome often associated with cancer and characterized by a depletion of visceral protein and lean body mass *(128)*. This syndrome afflicts approximately 50% of cancer patients and is responsible for 22% of cancer associated deaths. Cachexia differs greatly from simple starvation. Whereas starvation is associated with a decrease in body fat with a relative conservation of muscle mass, the cachectic state constitutes a great increase in the rate of whole-body protein turnover, inducing not only fat loss, but muscle loss as well *(129)*. Taken

together, these observations indicate that loss of skeletal muscle and therefore cachexia cannot be attributed simply to reduced food consumption.

Cytokines that are upregulated during cancer such as TNF-α, IL-6, and IL-1 have been implicated in cachexia *(128,129)*. TNF-α has been shown to reduce lipoprotein lipase activity in adipocytes, while parenteral administration of TNF-α induces anorexia, an increase in acute phase proteins, as well as protein and fat breakdown *(130,131)*. Administering antibodies to TNF-α increased food intake in an animal model of cachexia, but did not ameliorate weight loss *(132)*. In one study, circulating TNF-α was not detected in cachexic cancer patients *(133)*, however TNF-α production by peripheral blood mononuclear cells was elevated in pancreatic cancer patients with an acute phase protein response *(134)*. Acute phase protein synthesis is induced by a variety of proinflammatory cytokines, which may account for the different results seen in animal models and human studies regarding TNF-α alone. IL-6 is a potent inducer of the acute phase protein response. Elevated IL-6 levels can be found in some weight-losing cancer patients *(135)* and anti-IL-6 antibodies can downregulate the development of cachexia in animal models *(136)*. A similar response is also seen with IL-1 *(137,138)*.

The best way to treat cancer cachexia, logically, is to cure the cancer. Unfortunately, this is not a frequent achievement in adults with solid tumors and to date, nutritional intervention is considered one of the best ways to improve symptoms associated with cachexia. Fatty acids, particularly ω-3 fatty acids, have been shown to have anti-inflammatory benefits and as such are a potential treatment option for cachexia. An early clinical trial determining the effects of ω-3 PUFA on cancer patients fed 12 g of fish oil (18% EPA/12% DHA) reported a beneficial effect on weight gain *(139)*. A more recent study supplementing similar amounts of fish oil for 2 wk to patients with advanced cancer did not find an effect on appetite, tiredness, nausea, well-being, caloric intake, nutritional status, or function *(140)*. An international, multicentre, double blind randomized trial published in 2003 *(141)*, examined the effects of an ω-3 PUFA and antioxidant enriched oral supplement on weight loss and lean tissue loss in pancreatic cancer patients compared with a control supplement. The enriched supplement provided 6 g of fish oil/d and vitamins C and E at over four and eight times the recommended amount for healthy individuals, respectively. There was a linear relationship between change in lean body weight and enrichment of plasma phospholipids with EPA, indicative of greater protein accretion corresponding to the intake of ω-3 PUFA. Additionally, when body weight change was related to protein intake only patients consuming ω-3 PUFA enriched formulation showed a positive relationship. In this study the ω-3 PUFA were given in an immunonutrient mixture, and it is likely that the oxidant/antioxidant status also played a role in mediating these beneficial clinical effects. Unfortunately, none of these studies examined potential effects on immune function.

A novel study by Moses et al. *(142)* indicated that 2.2 g/d of EPA + 0.96 g/d DHA contained in a protein and energy dense supplement ameliorated weight loss and improved the quality of life of pancreatic cancer patients significantly better than the same supplement without EPA. The same group also found that serum IL-6 and excreted proteolysis-inducing factor were significantly reduced in the group that consumed EPA *(143)*. Gogos et al. *(144)* demonstrated that supplementing ω-3 PUFA (18 g/d) and vitamin E (200 mg/d) to malnourished cancer patients improved the

production of TNF, restored the CD4/CD8 ratio in blood and prolonged survival. In an earlier study, Gogos et al. *(145)* demonstrated that supplementation with fish oil (18 g/d) decreased the number of T-suppressor cells and effectively increased the T-helper/T-suppressor ratio in cancer patients with solid tumors. The results of these studies suggest beneficial effects of feeding ω-3 PUFA on immune function during cancer.

6. CONCLUSIONS

There is convincing animal data that decreasing the ω-6:ω-3 n-3 ratio of the diet by consuming more ω-3 fatty acids (particularly EPA and DHA) is of potential benefit in preventing colon and breast cancer. Part of this preventative health effect may result from the beneficial effect on anticancer immune defenses. There is a growing body of literature suggesting that decreases in the ratio of ω-6:ω-3 in the diet enhance immune function through a number of mechanisms including competitive inhibition of AA metabolism, alterations in membrane composition, modifications in cell signaling processes and changes in the expression of genes in immune cells. In more advanced cancer, there is evidence that supplementation with ω-3 PUFA, by consuming fish oil, down-regulates cachexia. Although at the present time, it is too early to make any clear recommendations regarding the precise ratio of ω-6:ω-3 PUFA in the diet to impact favorably on anti-cancer defenses, the available evidence should encourage public health authorities to design primary prevention campaigns to reduce the ω-6:ω-3 ratio of the Western diet by promoting increases in ω-3 PUFA consumption in populations at risk.

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21 ω-3 Fatty Acids in Physical and Mental Health and Disease

Abolghassem Djazayery and Shima Jazayery

Abstract

Basic and epidemiological evidence shows that ω-3 fatty acids—α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)—play important roles in the protection and promotion of health and prevention and treatment of several physical and mental disorders and diseases. They can favorably influence inflammatory intermediate production and gene expression, two basic physiological processes, which would, in turn, lead to desirable improvements in physical and mental health—from growth of the fetus and the infant to cognition in the elderly. They also have effective roles in the vascular and retinal health, asthma and allergy alleviation, and cognitive development in children, slowing age-related cognitive decline and cancer risk reduction. ωω-3 fatty acids are beneficial in cardiovascular disease (CVD) as well as diabetes mellitus. They may enhance vasodilator mechanisms in forearm microcirculation and help reduce blood pressure and bring about desirable changes in the blood lipid profile. Marine fish oil, the richest source of ω-3 fatty acids, can reduce plaque progression and risk of fatal coronary heart disease (CHD) events at a dose equivalent to 1 g EPA + DHA/d. In addition, clinical trials have shown that daily consumption of poseidonol–PUFA-ω-3 fatty acid family–may lead to increases in plasminogen activator and decreases in coagulation and the atherogenic index in type 2 hyperlipidemic diabetics with CVD. At least in some types of cancer, the ω-6:ω-3 fatty acid ratio may have a role in prevention and treatment, a low-ratio downregulating the cell adhesion/invasion-related molecules. There is some evidence showing that the cardio- and cancer-protective effects of ω-3 fatty acids may share common pathways—ionic channels, Ca homeostasis, PKC activation, and matrix metalloproteinase.

There are not many published reports on the role of ω-3 fatty acid in depression, Alzheimer disease, or schizophrenia. The available evidence indicates, however, that they may have desirable effects in the prevention/alleviation of these mental disorders as well.

Key Words: ALA; Alzheimer; blood lipid profile; blood pressure; cognition; CVD; depression; DHA; diabetes mellitus; exercise; EPA; fetus; fish oil; growth; infant; lipoproteins; schizophrenia; vision; ω-3 fatty acids.

1. INTRODUCTION

Available evidence shows that ω-3 fatty acids—α-linolenic acid (ALA: C18:3n-3), eicosapentaenoic acid (EPA: C20:5n-3), and docosahexaenoic acid (DHA: C22:6n-3)—play important roles in the protection and promotion of health and prevention and treatment of several physical and mental disorders and diseases. Preformed EPA and DHA are normal constituents of some foods (e.g., marine fish, soybean oil, and walnuts), but

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they can also be synthesized to a limited extent in the body by conversion of ALA. Regular intakes of sufficient amounts of ω-3 fatty acids—whether in the form of natural or enriched foods, or supplements—reduce risk of inflammation, thrombosis, atherogenesis, carcinogenesis, diabetes mellitus, and asthma. ω-3 fatty acids can influence favorably inflammatory intermediate production and gene expression, two basic physiological processes, which would, in turn, lead to desirable improvements in physical and mental health–from growth of the fetus to cognition in the elderly.

The balance between ω-6 and ω-3 fatty acids in the diet is vital, such that an altered ratio will interfere with normal physiological processes (e.g., immunity, and increase disease risk). The optimal ratio is believed to be around 1:1 to 1:4.

This chapter discusses briefly the basic and epidemiological findings that bear evidence to the beneficial roles of ω-3 fatty acids in physical and mental health and disease and, where known, the underlying mechanisms.

2. PHYSICAL HEALTH AND DISEASE

2.1. Exercise

Exercise may cause mechanical stress, local ischemia, increased free radical production, and oxidative stress in skeletal muscles. Inflammatory intermediates, such as cytokines, are produced *(1,2)*. The fat content of the diet may influence exercise performance *(3,4)*. Some investigators have been specifically interested in the effects of ω-3 fatty acids. According to Thomas et al. *(5)* ω-3 fatty acid supplementation (4 g/d) may reduce resting plasma TG concentration, but inhibit the beneficial effect of aerobic exercise on postprandial TG response after 3 wk. In one study it was revealed that dietary supplementation with 4 g/d EPA + DHA for 4 wk could increase the plasma total high-density lipoprotein cholesterol (HDL) and HDL2-C in active young men *(6)*. In that study, treadmill exercise increased not only the total HDL and HDL3-C but also the total low-density lipoprotein cholesterol (LDL). Combining the exercise and ω-3 fatty acid supplementation led to additional effects on the total HDL and LDL levels. ω-3 fatty acids may lead to a reduction in plasma fibrinolysis, as well as in systolic and diastolic pressures by 5 to 10 mmHg, respectively *(7)*, and exercise-induced bronchoconstriction *(8)*. They can also increase coagulation activity *(7)* and improve exercise-induced and endothelium-dependent forearm vasodilation in CAD patients, nitric oxide (NO) being involved in improvement of the blood flow *(9)*. On the other hand, a few researchers have reported no beneficial effect resulting from dietary ω-3 fatty acid or fish oil supplementation on endurance performance, fatigue, cytokines, or creatine kinase production *(2,10)*. Physical activity may *(11)* or may not *(12)* affect fatty acid metabolism.

2.2. Inflammation

Thies et al. *(13)* supplemented the diet of healthy subjects with different ω-3 and ω-6 fatty acids or fish oil for 12 wk and found a decrease in natural killer (NK cell activity resulting from EPA. Another group of investigators *(14)* reported an increase in phagocytic activity by fish oil (as well as by high-oleic acid sunflower oil) in healthy individuals, although an increase in the LDL susceptibility to oxidation also occurred. More recent work has shown that α-linolenic acid may decrease the inflammatory

burden in both normal and overweight/obese subjects *(15)*. Chen et al. *(16)* also showed that DHA could play an anti-inflammatory role by increasing cytokine production and cell adhesion molecule (CAM) expression in diabetic retina. The dose of ω-3 fatty acids may be an important variable in their anti-inflammatory potency *(17)*.

Reviews over the last few years suggest chemically important anti-inflammatory effects by ω-3 fatty acids in rheumatoid arthritis, asthma, inflammatory bowel syndrome, psoriasis, as well as cardiovascular disease *(18–20)*.

2.3. Growth and Vision of the Fetus and the Infant

2.3.1. Growth

There is evidence that increased ω-3 fatty acid intake during pregnancy and lactation may improve growth of the fetus and the infant subsequently. Lucas et al. *(21)* studied the effect of marine ω-3 fatty acids on the gestational age of Nunavik women (a sea food-eating population) and the anthropometric measurements of neonates born to them. In the neonates arachidonic acid was two times lower and DHA and ω-3:ω-6 ratio, as well as percent of ω-3 fatty acids (of total unsaturated fatty acids in the umbilical cord plasma phospholipids), three times those of southern Quebec neonates. Gestational age and birth weight were higher by 5.4 d and 77 g, respectively, in the third tertile of percent of ω-3 fatty acids, as compared with the first tertile. Another group of workers *(22)* reported an improvement in the ω-3 fatty acid status of both the mothers and their neonates, as determined by measuring DHA and EPA in maternal peripheral red blood cells (RBC) at week 37, resulting from fish oil supplementation from week 20 of pregnancy. The high levels of RBC remained high until 6 wk post-partum.

Fish oil supplementation in the first 4 mo of lactation has also been reported to increase, at the age of 2.5 yr, the body mass index (BMI) and waist and head circumferences of the children born. The BMI was positively associated with the maternal RBC DHA content *(23)*. Because the formation of DHA is restricted in infants with intrauterine growth retardation *(24),* supplementation of the preterm formulas with LCPUFAs (DHA and arachidonic acid) may also have an impact on the growth and body composition of pre-term infants, resulting in a higher lean body mass and a lower fat mass by the age of 1 yr than the respective control values *(25)*. One group of investigators, however, concluded from a cohort study conducted between 1999 and 2002 and involving 2109 women, an inverse association between seafood intake during pregnancy and fetal growth *(26)*.

Rrecent reviews have shown that both term and preterm infants may benefit from very long chain fatty acid (VLCFA) supplementation of mothers *(27),* whereas some researchers believe it is the preterm infant that benefits more *(28)*.

2.3.2. Vision

In a double-blind-randomized controlled clinical trial, the formula of 103 term infants was supplemented with DHA (0.36% of the total fatty acids) and arachidonic acid (AA, 0.72% of the total fatty acids) from day 5 *(29)*. The infants were followed up for 1 yr. Sweep visual evoked potential (VEP) acuity and random-dot stereo-acuity were better than in the control group up to weeks 52 and 17, respectively. Also, the RBC DHA content in the supplemented group doubled by week 17 and tripled by week 39. These findings confirmed results reported by other researchers, who had previously showed that baby foods containing DHA-enriched egg yolk (115 mg DHA/100 g egg

yolk) could improve visual function in breastfed term infants, the RBC DHA levels and VEP acuity being correlated ($r = 0.50$; $p = 0.0002$) *(30)*. One group of investigators, however, found no improvement in visual acuity in pre-term infants whose food had been supplemented with DHA and AA *(31)*.

DHA can have a role in the health of the diabetic retina due to its anti-inflammatory action, inhibiting cytokine-induced cell-adhesion molecule expression in retinal vascular endothelial cells *(16)*.

SanGiovanni and Chew *(32)* reviewed extensively the literature on the cytoprotectieve and cytotherapeutic roles of ω-3 fatty acids in angiogenesis and neuroprotection within the retina. Their conclusion was that ω-3 fatty acids may exhibit protective actions against age, inflammatory, ischemia, light, and oxygen associated pathology of the vascular and neural retina.

2.4. Asthma

The diet of a group of Australian pregnant women with a family history of asthma was supplemented with ω-3 fatty acid sources (tuna fish oil, ω-3 fatty acid-rich margarine, or cooking oil). Symptoms of asthma and atopy were assessed in the infants born to them at the age of 18 mo. Wheeze, bronchodilator use, and nocturnal coughing were significantly lower in children with high plasma levels of ω-3 fatty acids. The decrease in the serum IgE was not significant *(33)*. These findings were generally confirmed by Oddy et al. *(34)*, who showed that a diet with a higher ratio of ω-3:ω-6 ratio helped protect asthmatic children against symptoms of the disease. Stoney et al. *(35)*, however, found no protection against atopy in high-risk breastfed infants due to colostrum's high ω-3 fatty acid levels; in fact the risk increased. Another study showed a 10% reduction in the prevalence of cough in atopic children and a 7.2% decrease in sensitization to home dust mite allergen as a result of dietary supplementation with ω-3 fatty acids *(36)*.

Dunstan and Prescott *(37)* reviewed the evidence on fish oil consumption in pregnancy as a preventive method for reducing risk of allergic disease in infants. They concluded that maternal diet supplementation with this rich ω-3 fatty acid source could be an effective method to prevent development of allergic–and probably other immune-mediated–diseases in children.

So far no association between blood ω-3 fatty acids on the one hand and a lower risk of asthma or atopy in young adults *(38)* or allergic diseases in adults *(39)* have been reported.

2.5. Cancers

In a prospective cohort study *(40)*, involving 169 colorectal cancer cases and 481 matched controls, it was shown that the total, as well as the individual, ω-3 fatty acids (α-linolenic, EPA, DHA) reduced significantly risk of the disease in men (highest vs lowest quartiles); the association in women was not statistically significant. Based on the results of an in vivo trial by Eitsuka et al. *(41)*, it was concluded that ω-3 fatty acids might have an inhibitory effect on the activity of telomerase, which is increased in most cancer cells, in human colorectal adenocarcinoma cells, by downregulating its reverse transcriptase (hTERT) and *c-myc* expression via protein kinase C inhibition.

Concerning cancer of the pancreas, there is some evidence indicating the inhibitory effect of EPA on the cancer cell growth in humans, partly resulting from induction of

apoptosis associated with enzyme activation or suppression *(42)* and/or the anti-proliferative and apoptotic actions of DHA *(43)*. On the other hand, some investigators are of the opinion that the anti-cancer potency of ω-3 fatty acids may be attributed to modulation of tumor cell chmosensitivity and downregulation of *Her-2/neu* oncogene expression *(44)*, or increasing the synthesis of syndecan-1, a proteoglycan involved in cell-to-cell adhesion, growth factor signaling *(45)*. In the case of lung cancer, the ω-6:ω-3 ratio can have a possible role in prevention and treatment, a low ratio down-regulating the cell adhesion/invasion-related molecules *(46)*.

There is some evidence showing that ω-3 fatty acid supplementation may improve preoperative nutritional status and pre- and postoperative inflammatory and immune response in cancer patients undergoing surgery *(47)*. Two recent systematic reviews concluded no *(48)* or inconsistent *(49)* beneficial effects due to dietary supplementation with ω-3 fatty acids on cancer risk/prevention or on the survival and quality of life of cancer patients. More research is needed in this area.

2.6. Diabetes Mellitus

Woodman et al. *(50)* supplemented the diet of type 2 diabetic subjects with ω-3 fatty acids and observed desirable changes in the plasma lipid profile–decreases in triglycerides and increases in HDL-2. No changes occurred in the total LDLor HDL. Other workers have been interested in walnuts, a rich plant source of ω-3 fatty acids. Tapsell et al. *(51)* studied the effects of supplementing the diet of diabetic adults with 30 g walnuts/d for 6 mo. Their data showed increases in the RBC membrane ω-3 fatty acids, the PUFA/SFA ratio, the plasma HDL and HDL total cholesterol ratio, as well as a reduction in LDL as compared with the respective control values. All these changes were statistically significant and their magnitudes time-dependent. The data revealed no significant changes in body weight, percent body fat, or HbA1c. ω-3 fatty acids can bring about other desirable changes in type 2 diabetics. In a clinical trial, daily consumption of poseidonol–PUFA-ω-3 fatty acid family for 8 months resulted in a significant increase in plasminogen activator and decreases in coagulation and the atherogenic index in type 2 hyperlipidemic diabetics with CVD *(52)*.

EPA and DHA may differ with regard to some of their effects. A double-blind-placebo-controlled trial of parallel design showed that the antithrombotic effect of DHA is more pronounced than that of EPA. Relative to the placebo control values, only DHA caused a significant decrease in collagen aggregation and TXB2 in type 2 diabetics with treated hypertension *(53)*. Woodman et al. *(54)* also reported that a daily intake by type 2 diabetic women of 4 g EPA or DHA for 6 wk led to an increase or decrease, respectively, in the LDL particle size, as compared with the placebo (olive oil).

The results of a cohort study in Tanzania revealed cold water fish consumption to be inversely associated with the levels of plasma leptin independently from body fat content *(55)*. The authors also found a positive association between plasma leptin and insulin levels ($r = 0.46$; $p = 0.004$).

Several reviews have appeared in the literature in recent years about ω-3 fatty acids and diabetes mellitus. In one, the evidence for the beneficial effects of ω-3 fatty acids in type 2 diabetics–reduced CVD mortality, reduced blood pressure, improved plasma lipid profile, decreased platelet aggregability, and improved endothelial function–are discussed based on epidemiological and controlled trial findings *(56)*. A particularly

interesting finding discussed is that it seems that increased ω-3 fatty acid intake, if along with saturated fat intake, can reduce the risk of conversion of impaired glucose tolerance to type 2 diabetes in overweight individuals. Another review *(57)* discusses mainly the probable molecular mechanisms of the prevention of insulin resistance by ω-3 fatty acids in type 2 diabetics and the obese.

2.7. Cardiovascular Diseases

Many studies have been conducted aiming at determining the effects of ω-3 fatty acid food sources or supplements on the blood lipid profile. Rivellese et al. *(58)* fed 162 healthy subjects 3.6 g/d fish oil supplements. The supplements brought about statistically significant decreases in VLDL-cholesterol and triglycerides and increases in LDL-cholesterol. There was no change in the LDL particle size. The final conclusion was that a moderate dietary ω-3 fatty acid supplementation would result in the plasma fasting and post-prandial triglyceride levels, with increases in LDL-cholesterol. In another trial it was shown that a mixture of EPA + DHA (4 g/d) and GLA (2 g/d) could result in favorable changes in the blood lipid profile after 4 wk in health women—a decrease in serum TG and an increase in the serum phospholipids total ω-3 fatty acid content. Based on the PROCAM risk score *(59)*, the 10-yr mypcardial infarction (MI) risk would be reduced by 43% *(60)*.

Although the effects of EPA and DHA are, in most cases, similar, in one study DHA was found to be more effective than EPA in improving the blood lipid profile *(61)*. DHA (1.52 g/d) can bring about significant increases in HDL and decreases in TG and the TG:HDL ratio, and also favorable changes in the fraction of LDL-C carried by small, dense particles, although it may also bring about an increase in the LDL level *(62)*. It can also enhance vasodilator mechanisms in forearm microcirculation and help reduce blood pressure *(63)*. There is preliminary evidence that antioxidant vitamins may potentiate the effects of ω-3 fatty acids. According to Shidfar et al. *(64)* a daily dose of 500 mg vitamin C along with a 1 g EPA supplement may lead to a significant reduction in ApoA-1 and MDA.

The desirable changes in the plasma lipid profile resulting from ω-3 fatty acid food sources or supplements would decrease CVD risk. Clinical and epidemiological studies confirm this. Djousse et al. *(65)*, using logistic regression and a generalized linear model, studied a sample of 1575 individuals in the United States (US) National Heart, Ling, and Blood Institute Family Heart Study, matched for possible confounding dietary, socio-economic and life style variables. They found an inverse association between the total α-linolenic acid intake on the one hand, and the prevalence odds of carotid plaques, and well as with segment-specific carotid intima-media thickness on the other.

Increased dietary ω-3 fatty acid intake through consumption of ω-3 fatty acid-enriched eggs may promote anti-inflammatory and immunoregulatory processes, reducing the risk/progression of atherosclerosis and inflammatory disease as well as diabetes. Daily consumption of 2 ω-3 fatty acid-enriched eggs for 8 wk brought about reductions in hsCRP from $0.36 \pm 0.0.02$ to 0.31 ± 0.01 mg/dL ($p < 0.03$) and in the fasting plasma insulin level from 0.32 ± 0.03 to 0.30 ± 0.02 ng/mL ($p < 0.02$), in normocholesterolemeic, normoglycemic, and normotriglyceridemic young adults; no other changes occurred. The subjects had a very low ωω3- fatty acid intake initially *(66)*.

Many authors have reported on the beneficial effects of fish oil. Fish oil can reduce plaque progression and risk of fatal CHD events at a dose equivalent to 1 g EPA + DHA/d. A review by Hooper et al. *(67)* concluded that different source of ω-3 fatty acids–fish, plant sources, or supplements do not seem to differ in effectiveness with regard to CVD risk reduction in the general population. Furthermore, there may be synergy between ω-3 fatty acids and the B-complex vitamins, the latter decreasing the plasma total homocysteine *(68)*, whose high levels are another CVD risk factor.

Another recent review discussed the mechanisms of the desirable effects of ω-3 fatty acids in CVD risk reduction and concluded that the cardio- and cancer-protective effects of these fatty acids share common pathways–ionic channels, Ca homeostasis, PKC activation, and matrix metalloproteinase *(69)*.

3. MENTAL HEALTH AND DISEASE

3.1. Cognition

3.1.1. The Fetus and the Infant

In a clinical trial the DHA in RBC phospholipids (as % of total fatty acids) was measured in mothers at delivery and their newborn infants. The infants were then followed for up to 2 yr and tested periodically for development of attention, distractibility, and single-object exploration. The findings showed a positive link between DHA in infants and their mothers and cognitive development *(70)*. These results confirmed those reported by Helland et al. *(71)* who had shown that maternal ω-3 fatty acid intake during pregnancy and lactation has favorable effects in later development of the children. Another group of researchers *(72),* however, found no association between DHA (or arachidonic acid) status (measured in umbilical venous plasma and/or RBC phospholipids) of neonates and their cognitive development at 4 yr of age.

3.1.2. Adults

Studies have also been conducted in adults. Whalley et al. *(73)* investigated the effects of fish oil supplements on cognitive aging in a group of subjects born in 1936 followed up longitudinally. Their findings showed a higher cognitive function in fish-oil users at the age of 64 yr, a significant positive association between both the total RBC ω-3 fatty acids and docosohexaenoic acid/arachidonic acid ratio and cognitive function after adjustment for childhood intelligence quotient (IQ), and no significant difference as regards childhood IQ between fish-oil users and non-users. Fontani et al. *(74)* also showed that ω-3 fatty acid supplements can improve attention and physiological functions in healthy adults. Another group *(75)* found from a prospective cohort study involving the elderly (≥65 yr old) an association between fish consumption and a slower age-related cognitive decline after adjustments for possible confounding factors, such as age, sex, race, education level, physical activity, energy, and alcohol intakes. However, other researchers reported an inverse association between cognitive decline and the RBC membrane ω-3:ω-6 ratio in the aged *(76)* and a positive association between fatty fish and marine ω-3 fatty acid consumption and risk of impaired cognitive function in the middle-aged population *(77)*. A recent review *(78)* concluded that there is not sufficient evidence in the literature to draw definite conclusions on the effects of ω-3 fatty acids on cognitive function in normal aging or on incidence or treatment of

dementia, although some evidence indicates a possible association between ω-3 fatty acids and reduced risk of dementia. Further research is needed in this area.

3.2. Depression

There is evidence that depression, a growing problem globally, is associated with absolute or relative ω-3 fatty acid deficiency in the diet. As compared with healthy people, in depressed individuals, ω-3 fatty acid levels and/or the ω-3:ω-6 fatty acid ratios are low in the plasma and/or RBC membrane and/or adipose tissue, and the severity of depression has an inverse relationship with ω-3 fatty acid contents of these tissues *(79–81)*. On the other hand, epidemiological studies in several countries have revealed that in communities where consumption of fish is high depression prevalence is low *(82,83)*.

It seems that ω-3 fatty acids may influence the adrenergic and serotonergic neural transmissions both directly, through affecting cell membrane fluidity and involvement in the process of transmission into the cells, and indirectly through inhibiting inflammatory cytokine production *(84,85)*. A reduction in the neural transmission is an impaired aspect in the pathophysiology of depression *(86)*. In other words, depression is an immunologic-psychic-nervous disorder, in which overproduction of inflammatory cytokines plays the principal part *(87,88)* and ω-3 fatty acids can help in its prevention and alleviation by diminishing their production *(89)*.

Only a few clinical trials have been conducted so far aiming at determining the effect of ω-3 fatty acids in major depression *(90–93)*. The results have not been consistent. Thus, no definite conclusions have been obtained, although on the whole the available evidence indicates that ω-3 fatty acids can be effective in prevention/alleviation of depression. It is worth mentioning that in none of these trials monotherapy with EPA has been performed. A recent review shows that ω-3 fatty acids may be beneficial in mood disorder therapies, in the perinatal period in women, as well as for infant development *(94)*.

3.3. Schizophrenia

In a study it was shown that a daily dose of 2 g ethyl-EPA for 6 mo as an add-on treatment in schizophrenic patients increased the EPA content of RBC membranes fivefold (indicating compliance), resulting in inhibition of ADP-induced platelet aggregation. There was also a marked improvement in 5HT responsivity, which is inversely correlated with severity of the disease. It was concluded that the beneficial therapeutic action of EPA may be through modulation of 5-HT2 receptor complex *(95)*. Other workers have shown that ethyl-EPA consumption by schizophrenic patients for 12 wk is an effective, well-tolerated add-on treatment, resulting in a significant reduction in Positive and Negative Syndrome Scale total score and dyskinesia scores *(96)*. du Bois et al. *(97)* published a review in 2005 and concluded that there is a strong relationship between EPA status (as measured by the RBC membrane fatty acid composition) and normal neurotransmission, altered PUFA levels contributing to the abnormalities commonly observed in schizophrenic patients. They also found that the therapeutic mechanism of ω-3 fatty acids involves the serotonin receptor complex. Das *(98)* proposed a hypothesis concerning development of schizophrenia, which states that the disease has its origins in the perinatal period in genetically susceptible individuals, triggered by maternal infection (leading to overproduction of pro-inflammatory cytokines in the mother and the fetus). In such a situation interference with EPA metabolites will lead to a deficiency of

neuroprotective LCPUFAs. If this hypothesis is proved, then perinatal LCPUFA supplementation may help prevent schizophrenia in adulthood.

3.4. Alzheimer's Disease

There are not many published reports on the role of ω-3 fatty acid in Alzheimer's Disease. In a study using a simple linear design, Alzheimer patients took ethyl-EPA for 12 wk.

The only statistically significant change was a small improvement in carer's visual analog ratings of overall assessment of functioning. No changes occurred in the rate of decline of efficacy measures. The authors mentioned that a longer treatment period might result in observable improvements *(99)*. A more recent study showed that DHA could have desirable effects in Alzheimer's. According to Lukiw et al. *(100)* DHA supplements reduced amyloid-β secretion, followed by the formation of NPD1 (a DHA-derived docosatriene found to be reduced in the disease in hippocampal cornu ammonis). The authors reported brain cell survival promotion by NPD1 by anti-apoptotic and neuroprotective gene expression mechanisms.

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22 Dietary Prevention of Adult Macular Degeneration

ω-*3 Fatty Acids and Antioxidants*

Sheila Sedig and Ronald Ross Watson

Abstract

Adult macular degeneration (AMD) is the leading cause of blindness in those over 55 in the United States (US). Treatment of AMD is not very successful, therefore finding ways to prevent or slow the progression of the disease is important. The development of AMD is described in this chapter. Because the pathogenesis is thought to begin with hardening of the retinal arteries, dietary research has focused on fats and antioxidants that have been shown to be effective in decreasing the risk for cardiovascular disease.

Lowering total fat, saturated fat, and *trans* fats in the diet and improving the balance ω-3: ω-6 fatty acid ratio in the diet may help decrease inflammatory damage leading to AMD. The inflammatory cascade is described with emphasis on the role of ω-3 and ω-6 fatty acids. Docosahexaenoic acid (DHA) is an ω-3 fatty acid found in abundance in the retina; increasing intake of DHA, particularly from fish also decreases the risk of developing AMD. Antioxidants in the diet, particularly vitamins A, C, E, and zinc, may help decrease oxidative damage in the retina that could lead to AMD. Current recommendations for decreasing risk factors for AMD are listed.

Key Words: Adult macular degeneration; diet; nutrition; ω-3 fatty acids; ω-6 fatty acids; antioxidants.

1. INTRODUCTION

Adult macular degeneration (AMD) is the leading cause of blindness in the United States (US). It is estimated that it will reach epidemic numbers with the aging of baby boomers. There are approx 15 million people with AMD in the US. Of these, 1.5 million have the most severe form of AMD *(1)*. A new case of AMD is diagnosed every three minutes in the US *(2)*. The socioeconomic and emotional impact of AMD on individuals because of its reading and driving capability impairment is particularly high, making it an important public health issue. It is estimated that by the year 2020, there will be almost 3 million people with severe AMD in the US *(3)*. While many ways of treating AMD are under study, currently less than 1% of people with AMD are treated successfully. Increasing ω-3 fatty acids and antioxidants in the diet may play a role in the prevention of AMD or in slowing its progression.

AMD occurs primarily in people over the age of 55. The macula of the retina, the concentrated area of photosensitive cells which enables us to see colors and fine details, begins to lose function leading to blurred, distorted or lost vision in the center of the

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visual field *(4)*. Because the brain compensates so well for loss of vision in the early stages of AMD, diagnosis tends to be made only after much damage has occurred and is therefore difficult to treat successfully.

To understand the mechanisms of the damage that cause AMD, one must first understand the anatomy and physiology of the macula in the retina. The macula is a small portion of the retina in the back of the eye. The macula is responsible for central vision. The fovea is the sensitive portion of the macula; it is about the size of the period at the end of this sentence and is the focal point of damage in AMD. The retina lies on top of a network of blood vessels called the choroid, the microvasculature of the eye, which supplies oxygen and nutrients to the retina as well as removes waste products away from the retina. Between the retina and the microvasculature is a compact layer of fibers, called Bruch's membrane, which provide structural support for the retina and elasticity to help protect this delicate tissue from damage. Nutrients, oxygen and wastes travel unimpeded through Bruch's membrane in the healthy eye. The transfer of oxygen and nutrients into the retina is aided by small pumps in the retinol pigment epithelium (RPE)—a single layer of cells between the retina and Bruch's membrane.

As one ages, the small vessels of the microvasculature of the eye can harden, preventing waste products from the macula from being adequately removed and decreasing oxygen and nutrient supply to the macula. Waste material accumulates in Bruch's membrane in small pockets called drusen. As drusen accumulates, it causes the macula to bow upwards, further away from its blood supply, decreasing further the exchange of toxins, oxygen and nutrients. It is this debris accumulation, drusen, and lack of normal blood flow that cause AMD.

There are two forms of AMD: dry and wet. According to the Macular Degeneration Foundation there are 13.5 million Americans with dry AMD and 1.5 million with the wet form of AMD. Dry AMD is caused from this debris accumulation and decreased blood flow. The physical distortion of the retina from being pushed upward causes visual distortion and some loss of vision. As the lack of blood flow and decreased removal of debris continues, further bowing of the retina causes more visual distortion and vision loss. Ten percent of those with dry AMD will go on to develop the wet form of AMD: as the body tries to restore blood flow to the retina, blood vessels begin to grow up into Bruch's membrane. Drusen is then reabsorbed by these blood vessels. Unfortunately these vessels are not as strong as normal vasculature and begin to leak fluid into the pocket created from the reabsorbed drusen, pushing the macula upward. This resembles a blister formation. As images are projected onto this uneven and distorted surface of the retina, the image itself is seen as uneven and distorted. Eventually these new blood vessels break through the RPE and leak fluid into the space directly under the photosensitive cells. Further ballooning upward of the macula continues with blood leakage into the area, eventually causing scarring and severe vision loss. These are called disciform scars.

Because the pathogenesis of AMD is thought to begin with hardening of the arteries supplying the retina, it is believed that the same mechanisms that cause coronary atherosclerosis may lead to AMD. Thus looking at fats and antioxidants in the diet may help provide some preventive dietary measures for AMD just as these dietary factors can be modified to decrease heart disease risk.

It is also known that a particular type of fatty acid, docosahexaenoic acid, DHA, a long-chain ω-3 fatty acid, plays a role in visual development in utero and is abundant in

the retina *(5)*. This fatty acid is found in fatty fish, walnuts, flaxseed, olive, and canola oil. Because the retina is also exposed to the damaging effects of sunlight, which leads to oxidative injury, antioxidants in the diet have been promoted as a treatment for AMD as well *(6)*. In addition, it has been found that individuals with rheumatoid arthritis who have been on anti-inflammatory medications long term have much lower rates of AMD *(7)*. It is presumed that these anti-inflammatory agents protect the retinal vascular bed from oxidative injury. Such injury could cause drusen development and/or changes in the blood flow in the retina eventually progressing to AMD. Protection from this oxidative damage can decrease the risk of AMD.

2. FATS

It is well-documented that cholesterol, saturated fats, and *trans* fats increase cardiovascular disease (CVD) risk, while cis-, mono-, and poly-unsaturated fatty acids reduce CVD risk. Cho et. al, reviewed these dietary fats and their impact on AMD in a prospective study with data from the Nurse's Health Study and the Health Professionals Follow-up Study *(5)*. From a pool of 42,743 women in 1984 and 29,746 men in 1986 aged >50 yr with no diagnosis of AMD, they reviewed 567 individuals who developed AMD by 1996. They assessed dietary fat intake using food-frequency questionnaires.

Their extensive analysis revealed that the higher the total dietary fat intake the higher the risk of AMD. This association, however, appeared to be dependent upon type of fatty acids rather than total fat. There was a higher risk with higher intakes of linlolenic, saturated, and *trans* fatty acids and a lower risk with higher intakes of DHA, particularly from increased intake of fish. Thus, compared with CVD risk, saturated fats, *trans* fats, and monounsaturated fats were similar. The fact that a polyunsaturated fat, linolenic acid, however, resulted in a different risk factor profile for CVD vs AMD indicates that there are differences in the pathogenesis of these two diseases. Whereas polyunsaturated fats (PUFAs) have a protective effect in CVD, they were correlated with a higher risk for AMD. The authors hypothesize that PUFAs may increase the degree of unsaturation in the macula leading to more oxidative damage than with fatty acid profiles which are more saturated (i.e., have fewer double bonds).

Hageman et al. reported finding a specific genotype that increases the risk of developing AMD *(8)*. He also reports that the composition of drusen in AMD is remarkably similar to deposits found in other diseases, notably atherosclerosis, Alzheimer's and glomerulonephritis. It is thought that these chemicals constitute end-products of the inflammatory cascade (i.e., that local inflammation causes an immune response designed to ward off infection, phagocytize debris, and then return the body to normal functioning). In persons with this genotype the cascade is not fully realized, thus the body does not return to normal functioning, but simply continues the local inflammatory cascade leading to further debris accumulation, increasing drusen formation, then worsening vascular exchange in the macula.

These two studies taken together are of particular interest in light of dietary research that has emerged in the last two decades. The local inflammatory response usually causes oxidation of fatty acids in surrounding cell membranes. Direct oxidation of fatty acids by free radicals can also occur. When damage occurs in an area, small blood vessels respond by dilating, increasing blood flow to the area, and slowing blood flow in

the area. Surrounding endothelial cells become swollen; small plasma proteins, mainly fibrinogen, leak into the area; and various immune cells, particularly T-lymphocytes and macrophages, are drawn to the area. Immune cells then begin to secrete cytokines, chemical messengers that activate, coordinate, and regulate cell growth, repair, immunity and inflammation. Cytokines include the interleukins (IL), the interferons (IFN), and tumor necrosis factor (TNF). Cytokines signal the surrounding cells to produce enzymes that metabolize fatty acids in the adjacent cells' membranes. These classes of enzymes include the cyclooxygenase (COX) and 5-lipooxygenase (5-lipox) enzymes. The metabolism of these fatty acids forms eicosanoids—prostaglandins, leukotrienes, and thromboxanes. The proinflammatory eicosanoids escalate the whole inflammatory process while the anti-inflammatory eicosanoids inhibit this process. Inhibition of cytokines and eicosanoid production has become the mainstay of pharmacotherapy in treating inflammatory diseases, including the ill-fated COX inhibitors—rofecoxib and celecoxib—and the non-steroidal anti-inflammatory drugs (NSAIDS). As stated above, those on longterm anti-inflammatory medication have been shown to have lower rates of AMD. The fatty acid profile of our cell membranes, then, has a direct impact on this inflammatory response because ω–6 fatty acids tend to be proinflammatory, whereas ω-3 fatty acids tend to be anti-inflammatory (Fig. 1).

Our ancestral diets had an ω-6:ω-3 ratio of approx 1:1.6, but may have been as high as 3:1.7, therefore for optimum health a ratio that approaches from 1:1 to 3:1 is recommended *(9)*, but the typical American diet is closer to 16:1 *(10)*. ω-6 fatty acids are prevalent in animal meats, whole fat dairy products, and packaged, convenience foods (i.e., "junk" foods); foods abundant in the typical American diet, whereas ω-3 rich foods—fish, walnuts, flaxseed, olive, and canola oil—are more limited in the US diet.

Because ω-6 fatty acids are proinflammatory and ω-3 fatty acids are anti-inflammatory, this drives the whole inflammatory cascade toward inflammation rather than toward resolution of inflammation. This could explain the development of AMD: if the fatty acid profile of the cell membranes in the retina is one which promotes inflammation, more debris could be formed, creating accumulation in Bruch's membrane as drusen; while simultaneously stifling the return to normal function.

Because DHA is found in abundance in the retina, a decreased intake of this particular ω-3 fatty acid may lead to a decreased amount of DHA in the retina. This could cause a decreased anti-inflammatory response in the eye, a site of much ultraviolet light damage, as well as creating a deficit of the fatty acid available for retinal cell repair and synthesis. Both of these factors could lead to an increased risk of AMD.

It is interesting to note that while Klein, et al. *(11)* found no increase in systemic markers of inflammation in individuals with AMD, they did find increases in white blood cell count and decreases in serum albumin relating to the progression of AMD, once it was present. This could implicate inflammation in the progression, rather than the pathogenesis of AMD. They also report that about one-third of their population was on anti-inflammatory medications which may have decreased levels of serum markers of inflammation, prejudicing their results.

The research on fatty acids and AMD is relatively new, so much more needs to be done to more definitively answer the question as to the possible role of dietary fatty acids in the pathogenesis and progression of AMD. What role these fatty acids may play in the prevention of AMD also needs further study.

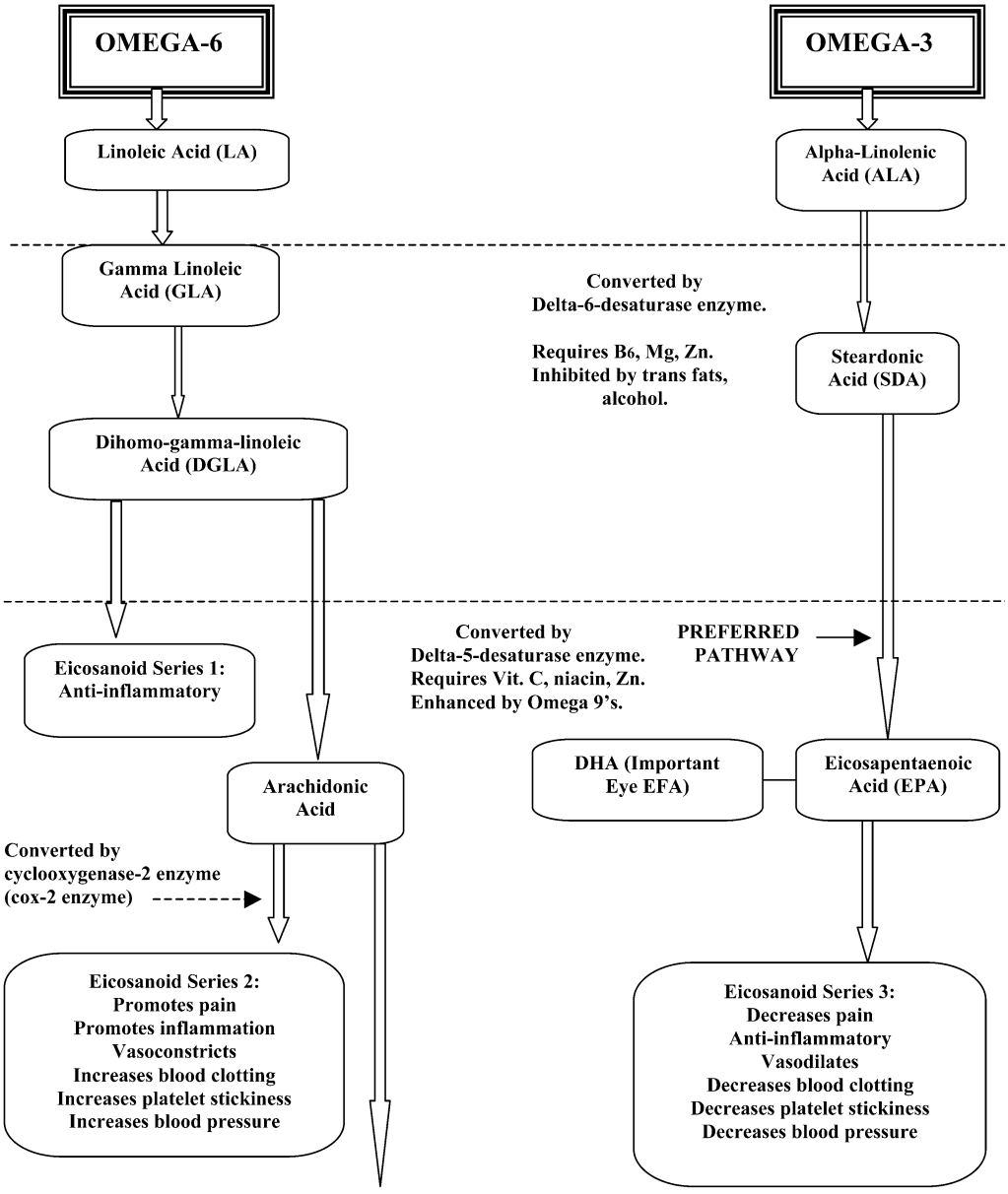

Fig. 1. Role of ω-3 and ω-6 fatty acids in inflammation.

3. ANTIOXIDANTS

It is well-established that vitamins A, C, and E are potent antioxidants in the body. They reduce free radical damage to tissues and prevent initiation of the inflammatory cascade secondary to this damage. In an excellent review, Donaldson lists not only the antioxidant qualities of fruits and vegetables, but also the protective vitamins, minerals, phytochemicals, and fiber constituents of these foods. He concludes with an outline for an anticancer diet, part of which recommends 10 servings of vegetables per day and 4 or more servings of fruits per day *(12)*.

The antioxidants vitamin A, several carotenoids actually, vitamin C, vitamin E, and zinc have been studied in AMD. In 1994, Seddon et al. reported on a 5-center study of dietary factors and development of AMD *(13)*. After controlling for smoking, a very high risk factor for developing AMD, and other risk factors, they found that the risk of AMD was 43% lower in individuals who consumed the highest levels of carotenoids, particularly lutein and zeaxanthin. The evidence for a positive effect of the vitamins C and E was not statistically significant. Of importance is that these vitamins were obtained from dark green, leafy vegetables rather than supplements; thus, the authors encourage the increased intake of lutein- and zeaxanthin-containing foods rather than supplements. Of particular interest is that the dominant pigments in the macula are these two carotenoids—lutein and zeaxanthin—which filter visible blue light, known to cause photosensitive cell damage. β-carotene and lycopene are found in limited amounts in the macula. This could explain why vitamins C and E seem to have a more positive impact on AMD than vitamin A in many other studies.

The Age-Related Eye Disease Study (AREDS) sponsored by the National Eye Institute did look at taking supplements containing high levels of the antioxidants β–carotene, the biologically active form of vitamin A, vitamin C, vitamin E, and zinc in persons who already have AMD *(14)*. These study subjects were followed for 6 yr.

This formulation: 15 mg β-carotene (25,000 IU of vitamin A), 500 mg vitamin C, 400 IU vitamin E, 80 mg zinc as zinc oxide, and 2 mg copper as cupric oxide to prevent copper deficiency, a condition that often occurs with high levels of zinc intake; did decrease the risk of developing advanced AMD by about 25%. The authors are careful to point out that this study was done with individuals who already had AMD, so there is no evidence that it may prevent the onset of AMD and they caution smokers to avoid large doses of vitamin A as it was shown in one well-publicized study to increase the risk of lung cancer in smokers.

A more recent study on antioxidants in AMD looked at β-carotene, vitamin C, vitamin E, and zinc; again from dietary sources rather than supplements. This study did look at individuals who had no evidence of AMD at baseline and followed them for approx 10 yr. Van Leeuwen et al. *(15)* found that there was a significant inverse relationship between the risk of developing AMD and the intake of vitamin E and zinc. This translated to an 8% risk reduction in AMD for vitamin E and a 9% risk reduction for zinc. Confounders such as smoking and atherosclerosis were adjusted for in this study. The authors relate that the dietary levels of these nutrients were far lower than the supplemented levels in the AREDS study, but this research does show a risk reduction of 35% for those individuals whose consumption of β-carotene, vitamins C and E, and zinc was above the median intake.

There is still much research to be done to give some hope to those with AMD or who are at risk for developing the disease. There are several new high-tech treatments for AMD, but if ways can be found to prevent the onset of the disease or slow its progression, much suffering and costly treatment could be avoided. There are already many reasons to change the typical American diet to prevent cancer, heart disease and diabetes. Obesity is even a risk factor for AMD. Because of this, public health professionals need to be continually diligent in informing the public about the health benefits of a low fat, higher ω-3 fatty acid, lower ω-6 fatty acid diet that is also high in antioxidants from fruits and vegetables.

Current recommendations that can be made for preventing or controlling AMD:

- Don't smoke.
- Eat at least 1 cup of dark green, leafy vegetables daily.
- Limit dietary fat and cholesterol; increase intake of ω-3 fatty acid foods.
- Maintain a healthy weight.
- Use alcohol in moderation.
- Exercise regularly.
- Wear a wide-brimmed hat and sunglasses when outdoors/in sunlight.

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23 Promoting Eye and Skin Health Through Intake of the Natural Carotenoid Lutein

Brandon Lewis

Abstract

Carotenoids in general are yellow, orange, or red lipophilic pigments that provide many of the colors found in nature. To date, over 600 carotenoids have been identified in nature and are produced by plants, algae, and bacteria. Animals appear to be incapable of biosynthesizing carotenoids, but many animals use them for a variety of purposes and therefore must obtain carotenoids from their diet. Two carotenoids receiving increased attention in the scientific literature are lutein and its isomer, zeaxanthin. In the human eye, lutein and zeaxanthin are concentrated in the macula and referred to as the macular pigment. Lutein is thought to function primarily in the eye as a filter of light, specifically blue wavelengths of light, and as an antioxidant. Large case-control studies indicating relationships between lutein, age-related macular degeneration (AMD), and cataracts were first published in the 1990s. In these studies, consumption of lutein and zeaxanthin was associated with a significant trend for decreased risk of AMD and cataract extraction. Since this time, relationships between dietary intake, serum concentrations, and macular pigment levels have been documented. The newest clinical research in subjects diagnosed with early AMD or cataracts indicates lutein supplementation after diagnosis may improve visual performance and augments earlier evidence that the dry form of AMD and cataracts may be responsive to changes in nutrition. As in the eyes, lutein is deposited in the skin. Lutein is thought to perform a similar function in the skin as in the eye, and epidemiological evidence, as well as animal studies, indicate a possible beneficial effect of supplementation. Nevertheless, a variety of questions remain unanswered. Future research should begin to confirm the potential of lutein to affect individuals already diagnosed with AMD or cataracts, and validate the beneficial effects of lutein on skin health in human populations.

Key Words: Lutein; zeaxanthin; eye health; skin health.

1. INTRODUCTION

Carotenoids in general are yellow, orange, or red lipophilic pigments that provide many of the colors found in nature. This includes plant and flower colorations as well as coloration of animals and birds. There are several subclassifications of carotenoids, with the most familiar being the pro-vitamin A carotenoids and the non-pro-vitamin A carotenoids (i.e., those that can and cannot be converted to vitamin A in the body). Carotenoids typically consist of 40 carbons or are derived from the $C_{40}H_{56}$ structure and contain a long linear-polyene chain made of carbons connected by alternating double bonds (*see* Fig. 1). The polyene chain is primarily responsible for the light absorption

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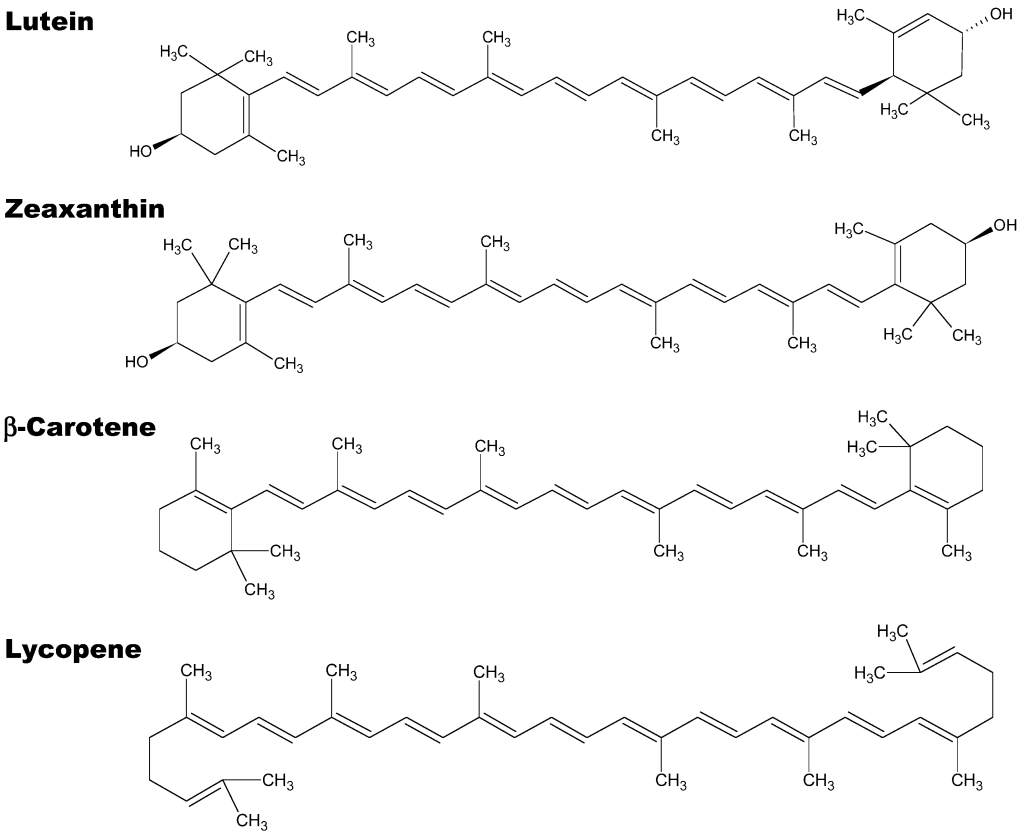
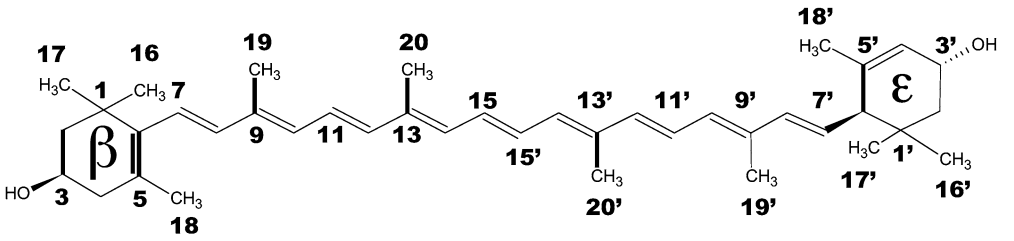


Fig. 1. Structures of common carotenoids.

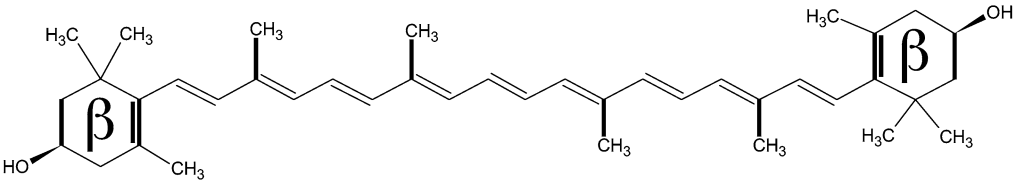
characteristics of the carotenoids; however, the end group attached to the polyene chain can influence the absorption maxima (A_{max}).

To date, over 600 carotenoids have been identified in nature and are produced by plants, algae, and bacteria. Animals appear to be incapable of biosynthesizing carotenoids, but many animals use them for a variety of purposes and therefore must obtain carotenoids from their diet. In humans it has been identified that approx 40 to 50 are typically consumed in the diet, but only between 14 and 18 primary carotenoids have been detected in serum indicating these carotenoids may have a specific function in humans *(1,2)*.

Two carotenoids receiving increased attention in the scientific literature are lutein and its isomer zeaxanthin. Lutein and zeaxanthin belong in the subclass of carotenoids known as xanthophylls, or oxygenated carotenoids. Lutein and zeaxanthin are considered oxygenated because of the free hydroxyl groups found on the rings of each molecule. These hydroxyl groups are responsible for the unique characteristics of lutein and zeaxanthin and allow them to orient within cell membranes and lipoproteins in ways other carotenoids cannot *(3–5)*. Lutein and zeaxanthin both consist of two rings connected by a polyene chain with alternating carbon double bonds. Their differences are very subtle and consist of the movement of one double bond in the ε ring from the 4′ to 5′



Lutein, (3R,3'R,6'R)-β,ε-caroten-3,3'-diol



Zeaxanthin, (3R,3'R)-β,β-caroten-3,3'-diol

Fig. 2. Structures of lutein and zeaxanthin.

position in lutein to the 5′ to 6′ position in zeaxanthin as well as different stereochemistry around the 3′-hydroxyl group (*see* Fig. 2).

Like all carotenoids, lutein and zeaxanthin are insoluble in water. Because of the hydroxyl groups on the 3 and 3′ carbons, however, the polarity of lutein and zeaxanthin is increased and allows them to insert in areas of the lipid membranes that are exposed to aqueous environments *(4)*. Lutein and zeaxanthin have limited solubility in some fats and oils but are most soluble in organic solvents such as methylene chloride and ethyl acetate.

2. SOURCES

The highest concentrations of free lutein in nature are found in the leaves of plants. In this role, lutein is an integral component of plant chloroplasts, working to accept excess energy from exposure to the sun as well as prevent oxidative damage *(6)*. The richest sources of free lutein in a typical human diet are dark green leafy vegetables, with the highest concentration found in kale followed by spinach (Table 1). Other vegetables and fruits contain smaller amounts of lutein compared with the green leafy vegetables. Researchers and government databases typically report the combined concentrations of lutein and zeaxanthin in analyzed foods (*http://www.nal.usda.gov/fnic/foodcomp/search/*). This results from the difficulty in separating these isomers when analyzing multiple carotenoids at once. Methods measuring lutein and zeaxanthin have been developed and are capable of separating the isomers so that quantitative analysis of lutein and zeaxanthin can be performed *(7)*.

3. ABSORPTION

Lutein is not manufactured by the human body and must be consumed in the diet. Once ingested, lutein is absorbed and deposited in the serum and other tissues. The

Table 1
Lutein/Zeaxanthin content of common foods

Food	*Lutein/Zeaxanthin (mg/100 g)*
Kale (raw)	40
Spinach (raw)	12
Broccoli (raw)	1.2
Corn (cooked)	1
Green Beans (raw)	0.6
Egg	0.3
Orange (navel)	0.1
Apple	0.03

absorption pathway of free lutein is similar to other lipophilic nutrients. Once released from the food matrix, lutein is incorporated into mixed micelles formed from bile salts released by the gall bladder. From the micelles, lutein is absorbed by the small intestine and incorporated into chylomicrons. The chylomicrons are transported to the liver where lutein is incorporated into lipoproteins for transport throughout the body in the blood *(8)*. Lutein and zeaxanthin are transported in the plasma predominantly in the form of high-density lipoprotein cholesterol (HDL), whereas other carotenoids are predominantly transported in the low-density lipoprotein cholesterol (LDL) fraction *(9)*. Once absorbed, lutein has been identified in several tissues of the body in significant quantities including the eyes, skin, breast, cervix, adipose, and brain *(10–13)*. The eyes have the highest concentration of lutein within the human body, specifically in the center of the retina, an area known as the macula lutea *(14)*.

The bioavailability of lutein has been compared in several matrices. Studies investigating the serum responses to lutein from green vegetables, spinach alone, and lutein supplements have indicated that lutein may be about twice as available from supplements than green leafy vegetables *(15,16)*. Castenmiller et al. found equivalent increases in serum lutein when subjects ($n = 72$) consumed various spinach preparations providing on average 11.5 mg lutein or lutein supplements providing 6.6 mg lutein *(15)*. Similar results were described by van het Hof et al. *(16)* when subjects ($n = 10$) consuming a low vegetable diet with a carotenoid supplement providing 12 mg lutein showed a significantly higher change in plasma lutein levels after 4 wk compared with subjects ($n = 23$) consuming a high vegetable diet providing 10.7 mg lutein (0.64 ± 0.05 vs 0.40 ± 0.03 µmol/L respectively; $p < 0.05$). This increase in lutein absorption from supplements may result from the body's difficulty in releasing lutein from the fibrous tissues and protein interactions within the plant.

Interestingly, whereas egg yolks have relatively low concentrations of lutein, they may be one of the most bioavailable sources because of the lack of chloroplast/chromoplast interactions and the high lipid and cholesterol matrix in the yolk *(17)*. Recently, Chung et al. compared the bioavailability of lutein from eggs or supplement *(18)*. Lutein provided naturally by an egg frittata (6 mg) or egg white frittata supplemented with lutein (6 mg) significantly increased lutein concentrations in the serum over the course of the study; however, between the two treatments, lutein provided naturally from the egg frittata significantly increased serum lutein levels compared with the egg white frittata supplemented with lutein (528.5 ± 56.3 nmol/L vs 288.1 ± 31.5 nmol/L respectively; $p < 0.05$).

4. SUGGESTED INTAKE

Regardless of source, the average daily dietary lutein and zeaxanthin intake in the United States (US) is approx 0.2 to 2 mg/d, and remains constant across gender and all age groups (NHANES 2001-2 Nutrient Intake Database; released 2004, CDC). Currently there is no explicit recommended intake for lutein. However, if the population was to eat according to the average recommendations of the Dietary Guidelines for Americans 2005, consumption levels could reach between 4 and 8 mg of lutein/zeaxanthin/d. Epidemiological evidence, animal models, and clinical data have indicated levels of 6 to 10 mg/d may be necessary to realize the health benefits associated with lutein consumption *(19–25)*.

Whereas the general intake of the US population is low, there are several sources available for increasing lutein intake. These include increased consumption of dark green leafy vegetables, corn, eggs, dietary supplements, and functional foods and beverages formulated with lutein.

5. MEASUREMENT OF LUTEIN AND ZEAXANTHIN

Lutein may have an antioxidant function as a free radical quencher in all the tissues where it is deposited. Similar to its role in plants, lutein may also absorb light in the visible spectrum in specific tissues (i.e., eyes and skin) *(26)*.

Lutein absorbs in the range of 400 to 500 nm with peak absorption at 446 nm. The ability of lutein to absorb light stems primarily from the conjugated polyene chain with a small interaction due to the ionone rings. Because zeaxanthin has 2 β ionone rings, the peak absorption is slightly higher at 451 nm.

Diet, blood, plasma, and serum levels of lutein and zeaxanthin are typically measured by ultraviolet (UV)/Vis spectroscopy following high-performance liquid chromatography (HPLC) separation. Tissue levels of lutein and zeaxanthin can be analyzed in a similar manner but require tissue biopsy and therefore are measured infrequently in living subjects. Tissue level data has primarily come from cadavers, animals, and as a by-product of other surgical procedures. As mentioned previously, researchers and government databases typically report the combined concentrations of lutein and zeaxanthin in analyzed foods, as well as in serum and tissues, as a result of the difficulty in separating these isomers when analyzing multiple carotenoids. However, methods exist that allow separation of lutein and zeaxanthin so that quantitative analysis of each isomer can be performed *(7)*. Two noninvasive methods have been developed and are commonly used to measure lutein and zeaxanthin in the eyes (macular pigment optical density [MPOD]): (i) heterochromatic flicker photometry and (ii) resonance Raman spectroscopy.

Heterochromatic flicker photometry has been adapted for MPOD measurement by exposing the retina to alternating reference wavelengths of light between 460 nm (blue wavelength at which macular pigment absorbs) and 540 nm (green wavelength at which macular pigment absorption is minimal). Initially, the subject observes alternating reference wavelengths as a flicker. The intensity of blue light is controlled by the subject and increased until the flicker is minimal or eliminated. Subjects with a higher MPOD will require a higher intensity of blue light to minimize or eliminate the flicker.

Resonance Raman spectroscopy directly measures the MPOD using lasers. When excited by light wavelengths between 450 and 550 nm, resonance Raman signals are emitted by lutein and zeaxanthin and detected by a Raman spectrometer. When adapted to the human eye, a 1-mm diameter argon laser generated beam at 488 nm is focused on

the foveal region of the retina for 0.5 s. Scattered light is then detected by a Raman spectrometer. Macular pigment optical densities can then be calculated by comparing the response to a calibration curve of known concentrations of lutein in solution.

6. LUTEIN IN EYE HEALTH

In the human eye, lutein and zeaxanthin are concentrated in the macula and referred to as the macular pigment. However, lutein has also been found in other structures in the eye including the peripheral retina, iris, retinal pigment epithelium/choriod, ciliary body, and lens *(14)*. Lutein is primarily thought to function in the eye as a filter of light, specifically blue wavelengths of light, and as an antioxidant. As a filter of blue light, it is estimated that the reduction in blue light reaching the macula (typically a 40% reduction) could significantly reduce the oxidative stress on the retina *(26)*. As an antioxidant, lutein may be able to limit the damage to the macula caused by free radical production within the body and by exposure to various wavelengths of light. Oxidation products of lutein and zeaxanthin have been detected in human and monkey retinas providing preliminary support of the antioxidant mechanism *(7)*.

The macula is in the center of the retina and contains a high concentration of cone receptors, which provides humans with maximal visual acuity. In the center of the retina (approx 0–0.25 mm), the ratio of lutein to zeaxanthin is approx 1:2 so that for every molecule of lutein in the central retina two molecules of zeaxanthin are found. Once out of the central retina into the peripheral retina (8.7–12.2 mm), the ratio reverses to 2:1 lutein to zeaxanthin. This change in ratio of lutein to zeaxanthin from the central to peripheral retina may indicate cell-specific association. In fact, the ratio of lutein to zeaxanthin in the central and peripheral retina is linearly correlated with the ratio of rods to cones in the central and peripheral retina *(27)*. In 1993, Bone and colleagues separated another isomer of lutein and zeaxanthin from the macula *(28)*. Using chiral column chromatography, the zeaxanthin fraction of the macular pigment was discovered to be equal amounts of (3R, 3′R)-β, β-carotene-3, 3′-diol (zeaxanthin), and (3R, 3′S)-β, β-Carotene-3, 3′-diol (meso-zeaxanthin). Considering that no meso-zeaxanthin was detected in plasma and that lutein levels in the plasma are higher than zeaxanthin levels, the authors hypothesized that meso-zeaxanthin may have been derived from retinal lutein.

A recent experiment in xanthophyll deprived monkeys has begun to support this theory *(29)*. Xanthophyll-free adult rhesus monkeys, which had been fed xanthophyll-free diets from birth, were supplemented with pure lutein or pure zeaxanthin for 4 to 12 mo. Macular sample analysis after supplementation with zeaxanthin revealed only zeaxanthin in the retina of the animals; however, analysis of macular samples after supplementation of lutein revealed both lutein and meso-zeaxanthin. This observation provides the first evidence that lutein may be converted in the eye to meso-zeaxanthin.

6.1. Age-related Macular Degeneration (AMD)

Lutein limits photo-oxidative damage in the eye passively by absorbing blue light and actively by quenching free radicals. Leading carotenoid researchers believe these functions may lead to a reduced risk of AMD and cataracts, shorten glare recovery times, and lessen chromatic aberration and photophobic response *(30)*. Ongoing research will lead to a more complete understanding of lutein's role in the eye.

The current interest in the macular pigment is the result of animal, observational, and clinical trials indicating an association between lutein and decreased risk for age-related macular degeneration. The early stages of AMD are characterized by the development of drusen in and atrophy of the macula followed by neovascularization and scarring in the late stages. Research published in 2004 by The Eye Diseases Prevalence Research Group indicated that approx nine million individuals age 40 and above in the US have been diagnosed with intermediate or advanced AMD *(31)*. In early AMD, also referred to as dry AMD, cellular debris deposits under the retina and forms drusen, which interfere with the normal function of the retina. Visual function can become affected including reduced contrast sensitivity, central visual field sensitivity, dark adaptation, and macular recovery compared to controls *(32–35)*. Late age-related macular degeneration, also known as wet AMD, often develops in areas where dry AMD exists *(36)*. Abnormal blood vessels grow and leak blood and fluid under the macula, causing scarring, which can lead to a rapid loss of central vision.

Large case-control studies indicating relationships between lutein and AMD were first published in the early 1990s. Researchers with the Eye Disease Case-Control Study analyzed the serum nutrients of 1036 subjects in a case control study of neovascular (wet) AMD *(37)*. The sum of the serum carotenoids analyzed, as well as the individual carotenoids (with the exception of lycopene) were inversely correlated with risk of AMD. The highest levels of lutein/zeaxanthin and β-carotene in the serum were associated with the lowest odds ratio of 0.3 ($p = 0.0001$ and $p < 0.0001$ respectively). Seddon and colleagues found similar relationships between dietary lutein/zeaxanthin levels and risk of AMD while investigating AMD risk and consumption of carotenoids and vitamins A, C, and E *(38)*. Consumption of lutein/zeaxanthin was associated with a significant trend for decreased risk of AMD (odds ratio = 0.43, $n = 876$, $p < 0.001$) in the highest quintile (5.8 mg lutein/zeaxanthin/d) compared with the lowest quintile (1.2 mg lutein/zeaxanthin/d).

In a Midwest population. the relationship between dietary intake, serum concentrations, and macular pigment levels was documented by Curran-Celentano and colleagues *(39)*. Dietary intake, serum lutein, and MPOD were measured in 208 healthy adult volunteers between the ages of 18 and 50. Dietary lutein/zeaxanthin, serum lutein, and serum zeaxanthin were significantly related to MPOD ($p < 0.005$) indicating that as dietary or serum levels of lutein and zeaxanthin increase, a corresponding increase in MPOD was observed. Subsequently, Broekmans et al. described a similar relationship between serum lutein and zeaxanthin levels and MPOD in men but not women *(40)*. Autopsy analysis of lutein and zeaxanthin levels in the macular pigment also indicated a significant inverse relationship between lutein and zeaxanthin concentrations in the macula and risk of AMD ($p < 0.0005$).

Human intervention studies have indicated that macular pigment can be modified by increased dietary intake of lutein through green leafy vegetables or by supplementation of lutein alone *(25,42–47)*. Studies supplementing healthy adult subjects with 10 to 30 mg lutein/d from food or supplements for up to 20 wk have all shown significant increases in mean MPOD *(44–47)*. However, it was not until 2004 that the largest clinical trial to date demonstrated that subjects already diagnosed with AMD could increase their MPOD through lutein supplementation *(25)*. This intervention study conducted at

a US Veterans Administration hospital evaluated the effect of supplementation (10 mg lutein alone vs 10 mg lutein with additional nutrients) in 90 mostly male subjects diagnosed with dry AMD. The subjects were supplemented daily for 1 yr. Consistent with previous studies, MPOD increased significantly in both supplemented groups (34% lutein alone and 43% lutein plus other nutrients, $p < 0.05$). This was also the largest study to show improvements in visual function with lutein supplementation. Specifically, glare recovery, visual acuity, and contrast sensitivity were all improved with lutein supplementation. This study augments earlier clinical evidence that the dry form of AMD may be responsive to changes in nutrition *(48,49)*.

6.2. Cataracts

A cataract is a natural clouding of the lens, the part of the eye responsible for focusing light and producing clear, sharp images. When lens cells die and accumulate they cause the lens to cloud, reducing the light to the retina, which can cause images to look blurred or fuzzy. For most people, cataracts are a natural result of aging; however, they may also occur as the result of disease or injury. Research published in 2004 by The Eye Diseases Prevalence Research Group indicated that approximately 20 million individuals age 40 and above in the US have been diagnosed with cataracts *(31)*.

Similar to the macula, lutein and zeaxanthin are the only carotenoids detected in the human lens, leading to the hypothesis that similar protective functions may occur in the lens as seen in the macula *(14)*. This is supported by both in vitro and epidemiological data. In vitro, human lens epithelial cells pretreated with lutein and zeaxanthin significantly decreased lipid peroxidation as well as c-JUN NH_2-terminal kinase and p38 activation (both involved in cascades that mediate cellular stress responses) in the cells after exposure to UVB irradiation *(50)*. Two large epidemiological studies were published in 1999 investigating dietary intake and risk of cataract extraction *(19,20)*. Chasen-Taber and colleagues followed a prospective cohort of female nurses (45–71 yr of age, $n = 77{,}466$) for 12 yr determining nutrient intake and risk of cataract extraction *(20)*. Increasing lutein + zeaxanthin intake from lowest to highest correlated significantly with a reduced risk of cataract extraction (2.0 mg/d vs 11.7 mg/d, RR = 0.78, $p < 0.05$). Similarly, Brown and colleagues determined a 19% reduction of risk of cataract extraction in 36,644 male health professionals (45–75 yr of age) as lutein + zeaxanthin intake increased from lowest to highest (1.3 mg/d vs 6.9 mg/d, RR = 0.81, $p < 0.05$) *(19)*.

Additional research on cataracts has correlated higher levels of lutein consumption with a decreased risk of cataract development. Lyle et al. determined that dietary intake of lutein + zeaxanthin were the only carotenoids significantly associated with a reduction of risk of nuclear cataracts in subjects participating in the Beaver Dam Eye Study ($p = 0.002$) *(51)*. Gale and colleagues determined supporting correlations between plasma lutein concentrations and decreased risk of posterior subcapsular cataracts *(52)*. In this study, subjects within the highest tertile of lutein intake had an approximate 50% reduction in risk of posterior subcapsular cataracts than individuals in the lowest tertile ($p < 0.05$). Similar associations were not determined for zeaxanthin in this study. Finally, a small clinical trial has shown improvements in visual acuity and glare sensitivity in people with age-related cataracts with lutein supplementation *(53)*. Subjects

(n = 17) diagnosed with age-related cataracts were supplemented with 15 mg lutein, 100 mg α-tocopherol, or placebo three times/wk for up to 2 yr. Serum concentrations of lutein and visual performance were significantly increased after supplementation of lutein. Serum levels of α-tocopherol were not significantly different after supplementation, however, visual function was maintained with α-tocopherol supplementation as compared to control in which visual function decreased.

7. LUTEIN IN SKIN HEALTH

An emerging area of research is lutein's role in skin health, primarily resulting from the fact that the skin, like the eyes, is constantly exposed to light and the environment. The mechanism of action is thought to be similar to that in the eye, with lutein protecting the skin by absorbing blue wavelengths of light and quenching free radicals that may be produced in the skin after exposure to light and environmental assaults *(54,55)*. Lutein has been detected in the skin *(10,56,57)*, and researchers have shown that significant correlations exist between plasma and skin levels of lutein *(58)* as well as epidemiological links with melanoma *(59)*. Several studies supplementing animals with lutein have begun to confirm the theory that lutein may play a beneficial role in skin health.

Taylor and colleagues were one of the first to show an anti-inflammatory activity of lutein upon chemically- and ultraviolet- (UVB) induced erythema in the skin of rats *(60)*. Lutein was found to reduce erythema following the application of the chemical irritant, 12-*O*-tetradecanylphorbol-13-acetate (TPA), to the skin of rats as well as reduce the epidermal thickness of the skin of the animals after exposure to UVB irradiation. Subsequent studies in mice have shown similar results. Gonzalez et al. investigated the effects of the ingestion of supplemental lutein on the acute effects of UVB exposure in hairless mice *(21)*. The animals were fed diets supplemented with lutein for 2 wk prior to acute irradiation with UVB. The amount of ear swelling, skin cell hyperproliferation, and apoptosis were all significantly decreased with lutein supplementation compared with control non-lutein supplemented animals. Granstein and colleagues again used the hairless mouse model to show significant reductions in reactive oxygen species, inflammation, and immune suppression in animals supplemented with lutein before exposure to UV light *(22)*.

To date, no clinical trial has been published investigating skin health in which subjects were supplemented with lutein alone. Currently, only two human studies have been published (by Morganti and colleagues) investigating the effect of combined antioxidant supplementation including lutein. The studies indicate that antioxidant supplementation including lutein may reduce reactive oxygen species *(61)* and lipid peroxidation in the skin while increasing skin lipid content and hydration *(24)*.

8. SUMMARY

Age-related macular degeneration and cataracts are the leading cause of blindness in the elderly US population with over 20 million Americans diagnosed with some form of the diseases. Evidence continues to build indicating a role of lutein and its isomer zeaxanthin in eye health. Increased consumption of lutein in the diet or by supplementation may reduce the risk of developing AMD and cataracts possibly through protection

of the macula and lens by absorbing blue wavelengths of light and quenching free radicals. Recent clinical trials supplementing lutein show improvements in visual function in subjects already diagnosed with AMD or cataracts. Similar roles are thought to occur with lutein in the skin, and epidemiological evidence combined with evidence from animal studies, indicate a possible beneficial effect. However, a variety of questions remain unanswered. Future research in eye health should begin to give an understanding of the transport and deposition of lutein and zeaxanthin in the macula and lens, confirm the potential of lutein to affect individuals already diagnosed with AMD or cataracts, and validate the beneficial effects of lutein on skin health observed in animal experiments for human populations.

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24 Calcium Phosphate
Nutrition in Prevention of Early Childhood Dental Caries

Rainy Dawn Warf and Ronald Ross Watson

Abstract

Early childhood dental caries is defined by its complex etiology of intersecting causations. Diet in combination with good oral hygiene, is the most prominently alterable component of this etiology.

Providing enough calcium phosphate in the diet of the pregnant and nursing mother and eventually in the weaning infant ensures that a prominent component (calcium phosphate deficiency) of the complex diagram that is dental caries can be eliminated, thus reducing susceptibility. This is now being taken a step further in a new model of dentistry that examines the mineral content of saliva, as well as bacteria, counts and uses a combination of bacterial control and mineral availability to re-mineralize early lesions and prevent more extensive decay before it emerges.

Key Words: Dental caries; infant; early childhood; nutrition; diet; calcium; calcium phosphate; prevention; treatment; *Streptococcus mutans*; re-mineralization.

1. INTRODUCTION

How can an otherwise healthy child who has never once eaten a piece of candy or a slice of cake and has only been lucky to grace this world for one year, be the victim of rotten teeth? Why is it that archeological evidence shows very low historical rates of tooth decay among hunter gathers of long ago contrasting with high levels among our contemporary medically advanced society? The answers to these questions lay in part in nutrition's role and it's affect on the complex etiology of dental caries.

Infant dental caries are a perplexing and far-reaching disease. They involve a multifaceted syndrome that afflicts a large fraction of the world, regardless of gender, age and ethnicity; although it does tend to affect to a greater extent those with a low socioeconomic status *(1)*. It is one of the most common childhood diseases. It impacts over 50% of all 5- to 9-yr-olds, and over three quarters of all teens by the age of 18. Sixty percent of all children have at least one filled primary tooth by the age of 2 *(4)*. It can cause pain and discomfort in its victims and requires massive physical intervention at a critical time of emotional and psychological development in a child. What makes this disease most puzzling is that it afflicts children before they may have been fully exposed to negligent flossing habits or over consumption of sweets, patterns of behavior that can be more directly linked to caries in adults.

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2. RISK FACTORS AND THEIR INTERSECTION: A COMPLEX ETIOLOGY

Early childhood dental caries is primarily distinguished by a complex etiology. It is difficult with so many variables to winnow out concrete individual factors that are solely to blame for its onset and rapid aggressive progression. Instead, dental caries are characterized by the intersection of many variables. Isolated factors have been identified which independently put an individual at risk, but it is only with the junction of those individual risk factors that dental caries develope.

Hazards influencing the development of dental caries include, but may not be limited to: environmental toxins, disease, medication, nutrition both pre- and postnatal, as well as genetic predispositions. "Genetics may sometimes explain a predisposition as the process of enamel formation, or amelogenesis, occurs predictably in tooth after tooth, generation after generation. The size, shape, shade, and even caries susceptibility of dental enamel can be passed from parent to offspring" *(4).* This genetic predisposition does not mean that other factors don't allow this predisposition to come to fruition, and there may be many cases that have no genetic characteristic at all. Factors such as poverty *(1)* that have been linked over and over again as a primary indicator, can only hint at possible genesis. Poverty is not causal in nature as a lack of resources is not related to disease *per se*. Looking beyond genetics, poverty is an indicator that hints at social and behavioral patterns that do have a contributory affect on this puzzling disease.

Despite the difficulty in pinning causality to one primary factor, evidence is emerging that can illuminate previous markers, such as economic status, linking it more directly to nutritional standing *(1)*. The importance of examining the role of nutrition as one influential factor in caries development results from its direct link to human behavior. It becomes the most easily alterable component in the etiology of this disease. For this reason, this chapter focuses on this element of the disease and it's implication in both prevention and cessation. With the assistance of public health experts, this information could be properly disseminated to advance research and education. Alternatives to the current dental model which includes surgical treatment could then be more accessibly developed and circulated. Primarily the importance of adequate calcium levels in the diet as a preventative measure and the possible use of calcium phosphate solutions with bacterial control systems for early intervention, cessation, and possible re-mineralization of initial caries lesions could become an alternate model for caries treatment.

2.1. The Role of Streptococcus mutans *in Caries Development*

Dental caries is an infectious disease. It is often transmitted from caregiver to child. The culprit in most cases is the virulent bacteria *Streptococcus mutans.* This acid producing bacteria etches away at the surface enamel eventually exposing the internal structure of the tooth to disease. This *S. mutans* bacteria is not the sole culprit, it must share the blame. Other factors influence the ability of these bacteria to take hold within its host. Not all children under the care of individuals with high levels of this bacterium become infected, and many people who harbor the bacteria may unknowingly manage to keep it under controllable levels in their mouth. Factors such as saliva flow, pH, immune system health, tooth maturation, and morphology all affect the degree to which the infected bacteria will attack the teeth *(2)*. *S. mutans* is especially detrimental as it has the unique ability to successfully colonize flat surfaces and under ideal environmental

circumstances reproduces especially rapidly. It feeds on carbohydrates and sugars and secretes as a waste product, acids. These acids de-mineralize the enamel, which eventually leads to tooth decay.

Nutrition has been historically ignored or given little mention as a factor in this process. The primary culpability of food in the development of dental caries has been aimed at physical contact. Sugary or carbohydrate substances provide a direct food source for the *S. mutans*, giving them the nutrition they need to happily reproduce and thrive in their acidic environment. This is accepted fact but does little to explain the vast variability in data among victims of dental caries. Carie susceptibility has been routinely blamed on breastfeeding practices and has led dentists to recommend early weaning. Previously referred to as baby bottle rot, it has now been renamed Early Childhood Caries (ECC). This name change reflects a change in the understanding of the development of the disease. Data associated with claims that breastfeeding was casual in nature had been over generalized and closer observation has shown it to have noncariogenic properties *(3)*. Breast milk's role as a primary food source for infants does, however, become important upon examination of the role general nutrition, specifically sufficient amounts of calcium phosphate plays as a variable in this complex scenario.

2.2. Nutrition and Its Influence on Caries Susceptibility

Guidelines have been established that argue the importance of good dietary habits during infancy and childhood and their affect of minimizing risk of caries development throughout life *(4,5)*. These guidelines outline the importance of diet in minimizing caries risk and argue that "optimal dietary habits for oral health are consistent with dietary recommendations for systemic health, growth, and development" *(4)*. Illustrating that caries development may be an indicator of overall poor nutrition. Despite the focus of this chapter on the role of calcium phosphate, it should be stated that general nutritional health also plays a role in caries development. Diet should be considered a dual risk factor, providing an on site food source for *S. mutans*, and leaving the body as a whole undernourished and more susceptible to disease.

Undernourishment has a twofold risk for the teeth. First, it weakens the immune system, leaving the body more susceptible to bacterial invasion, without the proper lymphocytes on hand to attack back. This can be seen in HIV infected children who have an unusually high rate of dental caries that increase with moderate to severe immune suppression *(6)*. In addition to creating a vulnerable immune system, nutritional deficiencies have the additional consequence of depriving the body of teeth-specific nutrients such as calcium phosphate and zinc, which are necessary for proper tooth formation and re-mineralization. They are essential in creating the actual makeup of the teeth. Without sufficient amounts of these crucial elements the body is left without the proper building blocks to create and maintain the teeth structure.

3. DEVELOPMENTAL HISTORY OF TEETH

The nutrition the developing embryo receives from its mother is fundamental in the development of teeth but occurs so early in pregnancy that many women may be unaware of their pregnancy or have not yet altered their long-standing consumption patterns. These early stages of infant growth are vital for tooth development; it is at this

juncture that tooth formation can be interrupted or mutated by external factors. Diseases, environmental contaminants, medications, and nutrition can all play important roles in the disruption of proper tooth mineralization *(7)*. Calcium phosphate may be critical in regulating tooth formation, although the mechanisms for such action are poorly understood *(8)*. Lack of calcium in addition to these other factors during significant stages may be responsible for pits and fissures in the enamel and thin and flakey surfaces that make easy entry for bacteria. The otherwise hard enamel surface becomes weak and often crumbly, sometimes to the extreme in which teeth literally crumble from the mouth.

Teeth, just like trees, have rings that illustrate this developmental history. A line known as the enamel stria indicates growth patterns of the tooth *(9)*.[1] A prominent striation known as the neonatal line is the indicator of interruption of the mineralization process at birth. This is one of the clearest indicators of nutritional factors affecting tooth development. This line shows a drastic change in environment. The infant feeding practices radically change from being automated through the umbilical cord to requiring the combined mother infant cooperation. This line in the dental formation record may indicate nutritional interruption during the first days of life when the nutritional needs of infants are often not met resulting from a delay in lactation from the mother and the learning curve for some infants in the nursing process. These changes in calcium availability are documented in the enamel stria.

The tooth is a complex structure. It is an intricate mineral rich cellular configuration containing connective tissues, nerves, and vasculature. During tooth development in utero, a protein based structural matrix is formed which is then mineralized. Teeth are made of minerals and thus need proper minerals to form, as well as ongoing supply of minerals to maintain their full health.

The tooth is comprised of several parts: enamel, dentin, and pulp. Both the enamel and dentin are comprised primarily of calcium phosphate. For this reason it makes sense that calcium phosphate would be required in sufficient quantities during periods of tooth development.

4. TOOTH IS TO BONE: A STRUCTURAL COMPARISON OF CORPOREAL MINERAL RESERVES

The tooth structure is similar to bone, and although much more complex in character a comparison is needed to fully understand the role that both teeth and bone play as corporeal mineral reservoirs for such minerals as calcium phosphate. These reservoirs are evolutionary safety nets needed to help our ancestors through periods of calcium deficiency.

The internal structure of the tooth formed of dentin is the most similar molecularly to the bone in the rest of your body. Examining bone structure helps to illustrate the similarities in both structure and susceptibility of disease in teeth and bone. It also illustrates the existing re-mineralization process and its potential for enhancement to promote repair of early caries lesions.

[1]Strandring S, Ellis H, Healy JC, Johnson D, Willliams A, editors. Gray's anatomy : the anatomical basis of clinical practice. 39th ed., Edinburgh; New York: Elsevier Churchill Livingstone, 2005:5–9.

Bone tissue is made and maintained by several types of cells: osteoblasts, osteocytes, and osteoclasts. These cells play different roles, creating new bone, maintaining bone health, and destroying worn-out stressed bone. These processes all involve the transfer of minerals, most importantly calcium phosphate, in and out of circulation in the body. When new bone is made, minerals from the blood are used to harden the protein and collagen matrix with minerals. When bone is destroyed minerals are released into the blood stream.

Normal bone is always undergoing remodeling. This destruction of old bone tissue and reconstruction of new bone is a balanced process that happens throughout life to maintain healthy bone. This equilibrium is maintained if blood supplies of calcium are adequate. The osteoblasts require calcium for this renovation process. As long as adequate blood levels of calcium are maintained the interchange of calcium through the blood is a constant balanced process; as blood levels drop the balance is lost and the osteoblasts are not capable of doing their job, thus leaving bones weak, unmineralized, and prone to breakage and disease.

The parathyroid glands are responsible for regulating the body's calcium levels. These small glands, which are embedded in the tissue of the thyroid gland, detect fluctuations in the level of calcium in the blood. When the levels drop, they secrete parathyroid hormone (PTH). This causes calcium to be released from the bones, more to be reabsorbed through the kidneys, and more to be absorbed from food in the intestines. If levels rise too much, PTH secretion is decreased and calcium levels return to normal again *(10)*. This process allows the body to use the bones and teeth as calcium reservoirs, creating the ability to borrow calcium from these sources during times of calcium depletion. If chronic deficiencies exist in dietary patterns, overtime the bones and teeth become calcium weak leaving them more susceptible to damage and less useful as a calcium reservoir *(1)*.

4.1. Re-mineralization and Structural Repair

Re-mineralization is an important factor in maintenance of both teeth and bones and is essential in structural repair and dental health. Teeth are constantly involved in a dynamic dance of demineralization and re-mineralization. The surface of the tooth is entering and exiting stages in which a combination of factors, including the mouth pH, available minerals in the saliva, and available blood levels of those minerals provide an environment that is either conducive to mineral loss or may allow for minerals to reattach themselves *(12)*. In this way minor carious lesions may go undetected and repair themselves through re-mineralization without note from either patient or dentist. The teeth are able to do this because they (especially their outer surface) are made up almost entirely of minerals: magnesium, sodium, chloride, iron, zinc, copper, and large amounts of calcium phosphate. They utilize these available minerals in the saliva to repair themselves when diet allows for increased calcium blood levels, which in turn increase available calcium in the mouth.

Just like bone, teeth demineralize when inadequate blood levels of amorphous calcium require additions from these mineral reservoirs within the body. The complex structure of the tooth is designed to withstand ebbs and flows in the availability of calcium. The re-mineralization process is an evolutionary byproduct of infrequent nutritional stability in hunter and gatherer societies. This built in repair mechanism allows

for re-mineralization when adequate mineral stores become available to the body. Through frequent monitoring of teeth health, calcium levels could be increased in the blood and saliva to promote this natural re-mineralization process.

5. HISTORICAL CONSUMPTION PATTERNS: IMPLICATIONS FOR TEETH AND BONE HEALTH

Historically hunter gatherer groups consumed diets substantially higher in calcium than current diets *(13)*. As hunter gathers didn't have access to milk products it is most likely that most of their calcium consumption came from animal bone and possibly soil elements, as clay consumption is still documented by anthropologists on many continents *(11)*. Worldwide dental caries rates climbed with the adoption of western practices. In the US alone over 95% percent of the population is afflicted with dental caries *(12)*. Contemporary life consists of diets that are not adequate for our bodies cellular needs; depleted by soaring consumption of nutritionally void snacks and little in the way of nutrient rich foods that provide important minerals. This diet is new to humans; it has emerged most extensively subsequent to the industrial revolution of 200 yr ago *(13)*. With this rapid change in nutritional consumption many chronic diseases have emerged. Bone and periodontal disease are only some of the chronic diseases that can be linked to dietary change over time. Osteoporosis and rickets may have emerged in part as a result of insufficient quantities of key elements, such as calcium phosphate, vitamin D, and zinc. Osteoporosis and rickets are common bone diseases that are directly correlated to inadequate absorption of vitamin D and calcium and have frequent implications for dental health *(14)*.

Although less well documented than osteoporosis and rickets despite the huge population involved, early childhood dental caries are also substantially affected by similar consumption patterns and in many cases parallels the development of bone disease. There is a strong association between rickets,— a softening of the bone—and infant dental caries, often occurring simultaneously in patients *(15)*. One could simplistically explain dental caries in some manifestations as rickets of the teeth. Severe dental caries may include softening of the dental surface and even crumbling of the teeth. Because both bone and teeth have structural similarities as well as comparable mineral requirements, rickets occurs when insufficient mineral levels are available to help solidify the collagen and protein matrix meant only as a building block within bone and not as a primary structure. It is in these ways that the link between bone health and dental health is easily made. The high parallel between bone loss and dental caries can most easily be linked to the important mineral that they share in common—calcium phosphate.

The evolutionary biology at a cellular level that provides the building blocks for our bodies has not fully caught up with the rapid cultural evolution that has shaped our diet so dramatically in the last 100 yr. This discrepancy in biological and cultural evolution may illuminate the important role that public health can play in behavior modification; with the ultimate goal of improving overall nutrition and thus increasing calcium consumption in people of all ages, assisting in the prevention of bone diseases in general and dental caries in particular. This could help escort a shift away from the surgical model of dentistry that has been the standard since the conception of modern dentistry.

6. CALCIUM DEFICIENCY: GUIDELINES FOR INCREASING CALCIUM CONSUMPTION

The National Institutes of Health (NIH) have prepared guidelines for calcium consumption needed to affect adequate health outcomes for bone, teeth, and immune function. They state that the preferred source of calcium is from calcium rich foods but that supplements are also useful in reaching optimal calcium levels. They go on to say: "A unified public health strategy is needed to ensure optimal calcium intake in the American population" *(16).* This call to action from the national institute of health highlights the obvious importance of calcium in the role of the prevention of dental caries.

This chapter has outlined the ways in which calcium phosphate affect the development of infant dental caries, including calcium phosphate's effect on the immune system and lack of mineral availability for proper teeth formation. It has also illustrated the process by which the body borrows calcium from the teeth during times of calcium deficiency. This process weakens the enamel, allowing easy entry of *S. mutans.* For this reason the best form of treatment of infant dental caries is prevention. This must come in the form of dietary changes which increase calcium consumption in pregnant and nursing mothers and promote calcium rich foods as early food choices for infants and toddlers. Calcium supplementation could also play a role and is usually agreed to be safe, and unlikely to pose any risks of adverse health effects to almost all individuals in the general population, as accidental overdose would have to involve unreasonably high levels of calcium. The UL for children and adults ages 1 yr and older (including pregnant and lactating women) is 2500 mg/d. Calcium requirements for the individual are greatly influenced by genetic variables and individual dietary inconsistency of calcium and other affecting nutrients making it difficult to generalize on individual calcium needs *(17)*. We do know, however, that calcium is something that the general population is not getting enough of. According to the Continuing Survey of Food Intakes of Individuals (CSFII 1994–1996), the following percentages of Americans are *not* meeting their recommended intake for calcium:

- 44% boys and 58% girls ages 6 to 11.
- 64% boys and 87% girls ages 12 to 19.
- 55% men and 78% of women ages 20+ *(18).*[2]

If 78% of women are not meeting their calcium requirements their developing fetuses and nursing children are directly affected by that deficiency and may in turn be deficient themselves. Table 1 illustrates the types of foods in which one can derive calcium, but the quantities needed for even small amounts of calcium are often far above the reality of current consumption patterns[2].

7. A NEW PREVENTION AND TREATMENT MODEL

Caries prevention through adequate calcium levels should be emphasized in current dental practice. Currently prevention is predominately emphasized through oral hygiene and sugar avoidance. Fluoride is the only mineral prescribed for supplementation. Most dentists and dental schools still do not outline the importance of adequate calcium levels as part of their standards related to prevention strategies.

[2]http://ods.od.nih.gov/factsheets/calcium.asp

Table 1
Calcium Content of Common Foods

Food	*Serving size*	*Calcium content*
Milk†	1 cup	300 mg
White beans	1/2 cup	113 mg
Broccoli (cooked)	1/2 cup	35 mg
Broccoli raw	1 cup	35 mg
Cheddar cheese	1.5 oz	300 mg
Low-fat yogurt	8 oz	300–415 mg
Spinach cooked‡	1/2 cup	120 mg
Spinach raw‡	11/2 cup	120 mg
Calcium-fortified orange juice	1 cup	300 mg
Orange	1 medium	50 mg
Sardines or salmon with bones	20 sardines	50 mg
Sweet potatoes	1/2 cup mashed	44 mg

Notes: †Low-fat milk has comparable or greater calcium levels than whole milk.
‡The calcium from spinach is essentially nonbioavailable.
This table was adapted from the American Academy of Pediatrics, Calcium Requirements for Infants Children and Adolescents.

The epidemiological discovery that fluoride in the diet and then experimentation with topical fluoride led to the dietary fluoride supplements in children, water fluoridation, and the use of topical fluorides in dentifrices, rinses, and gels. This was a huge public health strategy with far reaching effects. Fluoride acts as a bactericide and also replaces carbohydrates in the apatite, the calcium phosphate salt that makes up most of teeth. Teeth which are high in fluoride are much less soluble and therefore less susceptible to demineralization. This was a relatively new discovery given our history of dental practice and has drastically altered dental caries rates. It is outlined in all dental literature related to caries treatment and prevention. The use of calcium as a preventative and treatment strategy modeled after the success of fluoride has not yet trickled down from organizations like the national institute of health to effect dental practices.

The modern goal of treatment according to Graham Mount, DDS and Rory Hume, DDS from the UCLA school of dentistry "is now to change the local biochemistry so that the patient is no longer losing tooth mineral, so that the disease is then cured and the patient healed. This is logical, ethical, appropriate and achievable" *(19).* This model is only recently emerging. Doctors Hume and Mount predict that in the future this model of dentistry in which surgical treatment will be more accurately referred to as repair will expand to include bacterial diagnostics and mineral balance monitoring. In this way bacterial levels can be monitored along with calcium phosphate levels in the saliva. Measures can then be implemented to decrease bacteria levels through the use of various bactericides including natural measures like xylitol which has the effect of starving the bacteria population. Calcium levels can then be raised through supplementation and on site delivery. "These tools will mean that dentists will work to actively diagnose the disease at the molecular level before irreversible damage occurs, then treat the disease if it is present so that it will be cured, again before damage (i.e., cavities)

occurs *(19)*. They also predict that better re-mineralizing solutions will be developed as we begin to better understand the role that re-mineralization can play in restoring early dental caries lesions.

7.1. Calcium Phosphate and Its Role in Treatment

Research is just now starting to immerge as to possible treatment options with amorphous calcium phosphate applied directly to the teeth. The goal of this treatment is to elevate bioavailable calcium levels directly in the saliva and on the surface of the teeth creating an environment in which teeth are prone to re-mineralize surface caries lesions. This treatment is in the form of a cream that is placed in a dental cup or brushed directly on the teeth. This, with the addition of casein phosphopeptides (CPP) (which are milk derived components) "has a remarkable ability to stabilize calcium phosphate in solution and substantially increase the level of calcium phosphate in dental plaque *(20)*." This treatment currently is not being evaluated as a replacement for the surgical model of dentistry but does highlight a need for further examination into the various stages at which it is possible to promote re-mineralization and thus repair in the teeth using solutions such as the amorphous calcium phosphate.

Minimal evidence has yet to immerge as to the effectiveness of such a model in the face of severe late stage caries lesions. A Russian based study seemed alone in this exploration; they examined deep caries lesions and concluded there was a positive affect on these deep lesions. They showed that "tri-calcium phosphate normalized the function of the pulp, caused re-mineralization of dentin in the bottom of carious cavity, and were effective in the treatment of deep caries *(21)*." This is not enough evidence to promote this model in the treatment of late stage caries lesions but does highlight the need for further research.

Several studies *(22–24)* have found calcium phosphate affective in the repair of early caries lesions. This means that if the lesions can be detected when they are still small, calcium treatment could be an important alternative to drilling and filling the lesions, damaging the tooth structure and leaving it more vulnerable to future bacterial invasion. It is well known that no restorative material can adequately replace natural tooth structure for the long term, and for this reason preservation of natural tooth structure is important. "It is apparent that it is possible to re-mineralize and heal demineralized tooth structure to some degree. Therefore, neither enamel nor dentin should be removed simply because it has lost calcium and phosphate ions as a result of acid attack *(24)*." Re-mineralization has been shown successful in several random clinical trials that used mineralized mouthwash *(23)* and chewing gum *(25)* and in some cases examined the special interaction that the milk casein calcium phosphate has with fluoride to help improve overall mineral content *(26)*. Early lesions have been found to repair quite effectively using this calcium treatment. It has been shown to be even more effective when combined with fluoride. Early caries lesions seem to be very treatable in this fashion; which could save needless early drilling of teeth. The degree to which re-mineralization is possible and all the factors necessary to promote it effectively is still undetermined.

This treatment model is especially important in early childhood caries as it is much less invasive and could have the benefit of being less psychologically detrimental on the developing young mind. At an age when the only options for reconstructive dentistry involve forced restraint during the procedure in which the child often is quite terrified,

or sedation that is often done in a hospital and can take up to 2 h of general anesthesia with unknown effects on the developing mind. Any alternative that could be used to prevent this invasive procedure on a still developing child certainly merits investigation.

Young cells in other areas of the body have shown a much greater repair mechanism than that of adult cells and thus theoretically early repair in children could have even greater implications and potentially proceed with greater success. As the field of calcium phosphate therapy for dental lesions is a new an emerging market the effectiveness in children is still not well documented.

Many creams are now being sold directly to dentists with the intention of use as an at home prescription therapy; consumers can now enjoy calcium in almost every toothpaste they buy. The main ingredient in toothpastes that help with tooth sensitivity is, in fact, calcium phosphate. The majority of calcium treatment currently being used is in the form of over the counter toothpastes are in low doses *(27)*.

8. CONCLUSION: A NEW MODEL BEGINS WITH PREVENTION

Calcium seems an important component of the future of dentistry and could prove especially critical in the treatment of early lesions in children. More important still is the prevention of dental caries prior for the need of such a treatment.

It is clearly imperative to adequately utilize the accessible research on the use of calcium in prevention of early childhood dental caries. It is the role of the public health practitioner to assist in the education of parents, pediatricians, and dental professionals as to the importance of early childhood nutrition, and prenatal nutrition. Information regarding adequate calcium levels and assistance with meeting supplementation needs must be made available to the general public. With proper education programs, early childhood dental caries—the most prevalent childhood disease—could be vastly diminished if not almost completely prevented. Stepping into the new world of dentistry, a new model is clearly visible. This model would focus on early detection of high bacteria levels and utilize a combination of nutritional support for body and teeth in addition to bacteria management to promote an environment that encourages re-mineralization in the early stages of caries development. This combination of bacteria control and nutritional management could allow children to move into adulthood with bright smiles.

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III Functional Components in the Wild: Health Benefits

25 Natural Antioxidants in Land- and Marine-Based Wild-Type Food
Risk Reduction

Peter F. Surai, Ambrose J. Spinnler Benadé, and Brian K. Speake

Abstract

The relationship between health and food choices and the factors that determine our health have attracted the attention of scientists and health professionals for many years. However, during the last decade it has become obvious that the diet has a pivotal role in maintaining our health. It is generally accepted that natural antioxidants play important roles in risk reduction of many degenerative diseases, including two major killers of modern society cardiovascular diseases and cancer. In fact, a delicate balance between antioxidants and pro-oxidants in cells is an important determinant of various physiological processes and maintenance of this balance is the main aim of so called an integrated antioxidant system built in the human body. This system was developed during evolution to provide an antioxidant defence and give a chance for animals and humans to survive in an oxygenated atmosphere. It is now widely accepted that fruits and vegetables are important dietary components responsible for maintenance of good health. However, molecular mechanisms of protective effects of fruits and vegetables have not been fully elucidated. One of the attractive ideas is that various antioxidant compounds of fruits and vegetables are responsible for prevention of oxidative damage in the digestive tract.

Key Words: Antioxidants; vitamin E; carotenoids; flavonoids; ascorbic acid; health.

1. INTRODUCTION

The relationship between health and food choices and the factors that determine our health have attracted the attention of scientists and health professionals for many years. However, during the last decade it has become obvious that the diet has a pivotal role in maintaining our health. Therefore, in most developed countries, nutritional practice has changed focus from combating nutrient deficiencies to addressing nutrient requirements for maintaining good health throughout life. Indeed, collectively, cardiovascular disease (CVD) (including stroke), cancer, and diabetes account for approx two thirds of all deaths in the United States (US) and about $700 billion in direct and indirect economic costs each year *(1)*. They accounted for close to 1.5 million deaths in the US in 2001 *(2)*. The economic costs of cardiovascular disease, cancer, and diabetes in the US in 2003 were estimated to be $351.8 billion, $189.5 billion, and $132.0 billion, respectively *(3–4)*. It is generally accepted that natural antioxidants play important roles in risk reduction of many degenerative diseases, including two major killers of modern society cardiovascular diseases and cancer.

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2. ANTIOXIDANTS AS COMMON ELEMENTS IN IDEAL DIETS

The last 50 yr have been characterized by the understanding of the impact of nutrition and dietary patterns on health. Epidemiological findings, supported by animal studies, have led to recommendations that people should consume at least two servings of fruit and three servings of vegetable daily *(5)* in addition to at least two servings of fish weekly *(6)*. Whereas findings and reports such as these have had an impact on the type and quantity of the food that many of us eat *(7)* the majority of adults in developed countries fall well short of meeting healthy eating guidelines.

In the scientific literature, three major so-called "ideal diets" have received substantial attention over the last few years. They are Mediterranean, Japanese and hunter-gatherer diets *(8)*. The Mediterranean diet is associated with decreased rates of various diseases. For example, this diet, based on green and yellow vegetables, fruits, grains and olive oil, has been observed to have a protective effect against development of atherosclerosis. The Japanese diet reflects the highest life expectancy whereas the hunter-gatherer diet is based on a nutrient consumption similar to that of our ancestors during human evolution.

The evolution of man is connected with a life-style of hunting and gathering. The food sources (animal and plant) remained the same during this evolution, but the proportions of foods, preferences, preparations, and the attainability changed *(9)*. It has been suggested that the nutritional patterns of Palaeolithic humans influenced genetic evolution during the time period within which defining characteristics of contemporary humans were selected *(10)*. Because the human genome has not changed much since the beginnings of agriculture, genetically, humans remain adapted for a Palaeolithic dietary regimen. Such diets were based mainly on wild game, fish, and uncultivated plant foods. In comparison with Palaeolithic man, today our diet is characterized by substantial increases of some substances and decreases of others. For example, in comparison with the Palaeolithic period, current US intake of vitamin E decreased by 4 times (7–10 vs 32.8 mg/d), carotene by 2 times (2.05–2.57 vs 5.56 mg/d), and ascorbate by 6 times (77–109 vs 604 mg/d) *(11)*. In the history of mankind, anthropology shows that humans were gatherers of fruits and vegetables for their daily nutritional needs. This tradition has changed dramatically with the development of agricultural industries *(12)*. Furthermore, the relocation of people from rural areas to cities also decreased fruit and vegetable consumption. Therefore, in contrast with our ancestors who used to eat a variety of various wild plants in large amounts, today's diet includes a limited number of plants, consumed in limited amounts, and as a result is comparatively poor in antioxidant compounds *(11)*. In general it seems likely that the Palaeolithic diet contained quite high concentrations of various antioxidants, including vitamin E, carotenoids, ascorbic acid, flavonoids, and selenium.

The main advantage of the described diet is an adaptation of the human digestive system to most of those nutrients. This means that high efficiency of assimilation from the diet and distribution in the body could be a driving force in health promoting properties of various compounds, including antioxidants and phytochemicals. Possible implications of this kind of diet on the digestive tract need further investigations. Details of two other ideal diets are presented elsewhere *(13,14)*, but the main conclusion that can be drawn from analysis of "ideal" diets is that natural antioxidants are among the major players in these diets. Indeed, these three diets are different in composition but they have some similarities in terms of low saturated fat, high proportions of ω-3 fatty acids,

and high levels of naturally occurring antioxidants. It is generally accepted that antioxidant nutrients, especially those from food sources, have important roles in preventing pathogenic processes related to cancer, CVD, macular degeneration, cataracts, and asthma, and may enhance immune function *(15)*. Recent research on phytochemicals has changed a view on dietary factors affecting our health. In particular it seems likely that there are hundreds and even thousands of such factors and now we are dealing just with the top of iceberg. Based on this information a concept of the integrated antioxidant system in the human body was developed *(13,14)*. Indeed, all antioxidants in the body are working in concert as a team, which we call "the antioxidant system" and in this team every member has its own job to do and interactions between the antioxidants is a key for effective antioxidant defence and health-promoting properties of various antioxidants.

3. NATURAL ANTIOXIDANTS AND HUMAN HEALTH

The most important food related cause of disease is free radical overproduction. Free radicals are constantly produced in vivo in the course of the physiological metabolism in tissues. It is generally accepted that the electron-transport chain in the mitochondria is responsible for major part of superoxide production in the body *(16)*. Mitochondrial electron transport system consumes more than 85% of all oxygen used by the cell and, because the efficiency of electron transport is not 100%, about 1 to 3% of electrons escape from the chain and the univalent reduction of molecular oxygen results in superoxide anion formation *(17)*. About 10^{12} O_2 molecules are processed daily by each rat cell and if the leakage of partially reduced oxygen molecules is about 2%, this will yield about 2×10^{10} molecules of reactive oxygen species/cell/d *(18)*. An interesting calculation was made by Halliwell *(19)* showing that, in the human body, about 1.72 kg/yr of superoxide radical is produced. In stress conditions it would be substantially increased. Clearly, these calculations show that free radical production in the body is substantial and many thousand biological molecules can be easily damaged if they are not protected. The activation of macrophages in stress conditions is another important source of free radical generation. Immune cells produce free radicals and use them as an important weapon to destroy pathogens *(13,14)*.

Free radicals are implicated in the initiation or progression phase of various diseases, including CVD, some forms of cancer, cataracts, age-related macular degeneration, rheumatoid arthritis and a variety of neurodegenerative diseases *(20)* (Table 1). In general, it is widely believed that most human diseases at different stages of their development are associated with free radical production and metabolism. Normally, there is a delicate balance between the amount of free radicals generated in the body and the antioxidants to protect against them *(13,14)*. However, an excess of free radicals, or lack of antioxidant protection, will shift this balance producing oxidative stress. Food components can modulate this balance and may thereby influence the rate of aging *(21)* as well as disease resistance of the human *(5)*. The most important step in balancing oxidative damage and antioxidant defence in the human body would be to enhance the antioxidant capacity by optimising the dietary intake of antioxidants through, for example, increased consumption of antioxidant-rich foods. These could be foods natural rich in antioxidants as in the case with some fruits and vegetables *(5)* (Table 2) or through modification as with so-called modified or functional foods.

Table 1
Free Radical Involvement in the Development of Human Diseases*

- Liver
  - Reperfusion
  - Toxic effects of chemicals: halogenated hydrocarbons, quinones, iron, acetaminophen, ethanol
  - Endotoxin
- Kidney
  - Autoimmune nephrosis:inflammation
  - Toxic effects of chemicals: aminoglycosides, heavy metals
- Lung
  - Normobaric hyperoxic injury
  - Bronchopulmonary displasia
  - Toxic effects of chemicals: paraquat, bleomicin
  - Emphysema
  - Asbestosis
  - Idiopathic pulmonary fibrosis
- Heart and cardiovascular system
  - Atherosclerosis
  - Hemochromatosis
  - Reperfusion: after infraction or transplant
  - Selenium deficiency (Keishan disease)
  - Toxic effects of chemicals: ethanol, doxorubicin
  - Myocardial infarction
- Gastrointestinal tract
  - Reperfusion
  - Toxic effects of chemicals: nonsteroidal and anti-inflammatory agents, alloxan, iron
  - Pancreatitis, Colitis, Intestinal ischemia, Gastric ulcers
- Blood
  - Malaria
  - Various anemias
  - Protoporphyrin photooxidation
  - Toxic effects of chemicals: phenylhydrazine, primaquine and related drugs, sulfonamides, lead etc.
  - Favism
  - Fanconi's anemia
- Eye
  - Retinopathy of prematurity
  - Photic retinopathy
  - Macular degeneration
  - Ocular hemorrhage
  - Cataracts
- Muscle
  - Muscular dystrophy
  - Overexercising
- Skin
  - Radiation (UV or ionizing)
  - Thermal injury
  - Toxic effects of chemicals: tetracyclines stimulating photosensitization
  - Contact dermatitis
  - Porphyria
- Brain and nervous system
  - Parkinson's disease
  - Alzheimer's disease
  - Tardive dyskinesia
  - Neuronal ceroid lipofuscinosis
  - Neurotoxins
  - Hypertensive cerebrovascular injury
  - Allergic encephalomyelitis
  - Multiple sclerosis
- Inflammatory-immune system
  - Glomerulonephritis
  - Vasculitis
  - Autoimmune disease
  - Lupus erythermatosus
  - Rheumatoid arthritis
- Miscellaneous/general
  - Aging
  - AIDS, Cancer, Diabetes
  - Inflammation
  - Trauma
  - Ischemia/reperfusion
  - Radiation injury
  - Toxic effects of chemicals: alloxan (diabetes), iron overload
  - Acute pancreatitis, Amyloidosis

*Adapted with permission from ref. *13*.

Table 2
Natural Food Sources of Some Antioxidants

Compounds	*Source*
Vitamin E (tocopherols and tocotrienols)	Oilseeds, vegetable oils, nuts, whole grains, cereals, margarine.
Vitamin C	Fruits and vegetables, berries, citrus fruits, green papers.
Carotenoids	Dark leafy vegetables, carrots, sweet potatoes, tomatoes, apricots, citrus fruits, kale.
Flavonoids/isoflavonoids	Fruits and vegetables, oilseeds, berries, peppers, citrus fruits, tomatoes, onions.
Phenolic acids/derivatives	Oilseeds, cereals, grains.
Catechins	Green tea, berries, certain oilseeds.
Extracts/essential oils	Green tea, rosemary, sage, clove, oregano, thyme, oat, rice bran.

Adapted with permission from ref. *123*.

3.1. Vitamin E

Vitamin E is the main biological chain-breaking antioxidant, found in food in the form of 4 tocopherols and 4 tocotrienols. Biological activity of vitamin E in tissues results mainly from α-tocopherol, but in food the main form of vitamin E is γ-tocopherol. It is possible that the gut is a special place for γ-tocopherol and tocotrienols to play their antioxidant role. Vitamin E is not stable and is easily oxidized during food processing. Synthetic antioxidants added to the food can inhibit vitamin E oxidation. Vitamin E (α-tocopherol) in the tablet or capsular form is mainly produced in the stable esterified form or as a mixture of tocopherols. The major vitamin E sources in the diet are vegetable oils (e.g., wheat, soybean, sunflower, and corn) and some other plant-derived foods (Table 3). For example, in middle-aged Japanese, vitamin E was mainly of vegetable origin with main contributions coming from spinach, safflower oil, and pumpkin *(22)*. In the United Kingdom (UK), the average daily vitamin E intake is 11.7 mg in men and 8.6 mg in women. Similar consumption was reported in the US and other countries with margarines and mayonnaise supplying 23% of total vitamin E consumed. These levels are in the line with the recommended daily allowances (RDA). In fact the Food and Nutrition Board of the Institute of Medicine recently published dietary reference intakes for vitamin E, which is 15 mg for adults being 50% greater than the generous allowance in the 10th edition of Recommended Dietary Allowances published in 1989. It has been concluded that, according to the RDA, the intake of antioxidants is adequate in healthy subjects *(5)*. However, the recent data of Bodner et al. *(23)* indicate that vitamin E intake in Scotland is 6.6 mg/y for women and 7.3 mg/d for men, comprising only 50% of the RDA and being lower than that in other European countries. In addition, there are several categories of people whose vitamin E consumption is lower than the RDA.

Vitamin E deficiency is associated with a development of a range of specific diseases involving major tissues of the organism including immune system incompetence, impairment of lipid metabolism, fertility problems, and increased susceptibility to common and specific diseases. There are also several clinical conditions where vitamin E deficiency

Table 3
Vitamin E Content of Some Plant-Derived Foods

Product	*Vitamin E activity as α-TE*[a]
Vegetable oils	
Canola	21.5
Coconut	0.7
Corn	19.8
Cottonseed	42.8
Olive	12.0
Palm	33.5
Palm kernel	6.2
Peanut	15.2
Safflower	34.9
Sesame	16.5
Soybean	17.1
Sunflower	49.2
Walnut	63.6
Wheat germ	173.6
Vegetables, fruits and berries	
Potato	0.05
Carrot	0.37
Broccoli	0.69
White cabbage	0.04
Lettuce	0.66
Spinach	1.22
Tomato	0.68
Sweet paper	2.21
Apple (flesh only)	0.24
Orange	0.36
Banana	0.21
Peach (flesh only)	0.96
Blackcurrant	2.30
Bread	
French	0.38
Rye	0.52
White	0.06
Margarine, Stick	
Soybean	9.0
Corn	20.9
Sunflower	25.9
Dressings	
Blue cheese	11.2
French	9.8
Italian	10.8
Mayonnaise	7.4

[a]mg α-tocopherol equivalents/100 g product. Adapted with permission from ref. *124* and *125*.

states are described: vitamin E malabsorption syndrome, abetalipoproteinemia, chronic childhood cholestasis, cystic fibrosis, total parenteral nutrition, and prematurity *(24)*. Recently, it has been suggested that detrimental consequences of vitamin E inadequacy could be a result of changes in gene expression. For example, it has been shown that vitamin E-deficiency induces significant molecular regulatory properties in liver cells with an altered expression of both antioxidant-defense genes and genes that control the cell-cycle and demonstrate that liver nuclear factor (NF)-κB activation is involved in this response *(25)*. Therefore, it is important to maintain an adequate vitamin E consumption not only to prevent liver oxidative damage but also in modulating signal transduction. Recently, it has been shown that vitamin E is able to directly influence gene activity and potentially can affect drug metabolism in humans *(26)*. In fact, it has been found that vitamin E potently regulates the expression of about 230 genes, functioning in metabolism, cell-cycle progression, and transcriptional regulation *(27)*.

Vitamin E can not be synthesized in the human and its adequate intake relies upon adherence to a well balanced diet. It has been suggested that by enhancing the intake of vitamin E by fortification of foods or by dietary supplements it may be possible to reduce the risk of many common, yet disabling human diseases. Furthermore, there are many studies suggesting that intake of vitamin E in amounts much higher than RDA are associated with reduced risk of various diseases *(5)* and with enhancement of certain immune responses *(28)*. Results from large-scale human observational studies suggest that antioxidant consumption reduces the risk of developing cardiovascular disease. However, the American Heart Association (AHA) maintains that there are insufficient efficacy data from completed randomized trials to justify population-wide recommendations for use of vitamin E supplements in disease prevention *(29)*. It is interesting that about one half of American cardiologists take supplemental vitamin E *(30)*. Even so, results of clinical trials with vitamin E supplementation were not as successful as expected.

During the past decade, the health benefits of vitamin E have been shown in several epidemiological studies *(21)*. For example, epidemiological evidence shows a lower incidence of infectious disease in subjects with high plasma tocopherol concentrations *(31,32)*. In this respect, Lachance *(33)* has shown that the optimal daily antioxidant intakes are 23 mg for vitamin E and 3.2 mg for carotene.

It seems likely that there are important differences in molecular mechanisms of action of various tocopherols and tocotrienols. Indeed, the unique vitamin action of α-tocopherol, combined with its prevalence in the human body and the similar efficiency of tocopherols as chain-breaking antioxidants, led biologists and health professionals to almost completely discount the "minor" tocopherols as topics for basic and clinical research *(34)*. However, recent discoveries have forced a serious reconsideration of this concept. For example, new and unexpected biological activities have been reported for γ-tocopherol which are not related directly to their chemical antioxidant activities but showing anti-inflammatory, antineoplastic, and natriuretic functions possibly mediated through specific binding interactions. Furthermore, a great body of epidemiological evidence suggests that γ-tocopherol is a better negative risk factor for certain types of cancer and myocardial infarction than is a α-tocopherol *(34,35)*.

Vitamin E is considered to be not toxic for humans and a daily dosage of 100 to 300 mg vitamin E can be considered harmless from a toxicological perspective and therapeutic

vitamin E doses start at several hundred mg/d and end at approx 1600 mg/d *(36)*. Clearly, vitamin E can be considered as a main contributor to the antioxidant potential of the digesta.

3.2. Carotenoids

Carotenoids recently were included into family of natural antioxidants. They exhibit their maximum antioxidant activity at low-oxygen pressures, which prevail in healthy tissues. It has been recently hypothesized that carotenoids are not the major antioxidant players themselves but rather are an important part of the antioxidant system *(13)*. Therefore antioxidant interactions including their recycling provide an effective and reliable system of defence from free radicals and toxic products of their metabolism.

Carotenoids comprise a family of more than 600 compounds responsible for a variety of bright colours in fall leaves, flowers (e.g., narcissus, marigold), fruits (e.g., pineapple, citrus fruits, paprika), vegetables (e.g., carrots, tomatoes), insects (e.g., ladybird), bird plumage (e.g., flamingo, cock of the rock, ibis, canary), and marine animals (e.g., crustaceans, salmon) *(37)*. These pigments provide different colours from light yellow to dark red and, when complexed with proteins, they can produce green and blue colorations. Yellow, orange and green fruits and vegetables provide a range of carotenoids.β-carotene, α-carotene, and β-cryptoxanthin are the major provitamin A carotenoids in human and lutein, zeaxanthin, and lycopene are major carotenoids in the diet which are not converted to vitamin A. Biological functions of these natural pigments in relation to animals or humans are not well defined but their antioxidant properties seem to be of major importance. In mixture with other antioxidants they could be much more effective than on their own, and the GIT could be a major place for these compounds to exert their activity. In some conditions, carotenoids can be prooxidants. However, it is well recognized that this possibility is not likely to be the case in physiological conditions, including in the GIT when an array of other antioxidants is present.

There are species and tissue-specificity in carotenoid actions. For example, tomatoes are rich sources of lycopene, an antioxidant carotenoid reported to be a potent singlet oxygen-quenching agent. In addition to its antioxidant properties, lycopene shows an array of biological effects including cardioprotective, anti-inflammatory, antimutagenic, and anticarcinogenic activities *(38,43)*. Whereas a great body of experimental evidence has been accumulated to demonstrate the potency and nature of the biological effects of carotenoids, in most cases their underlying mechanisms of action remain uncertain. This is the result of a range of biological effects observed and their tissue specificity, time- and dose-dependency, and limitations of the available model and delivery systems *(39)*. For example, the anticancer activity of lycopene has been demonstrated in both in vitro and in vivo studies. The mechanisms underlying the inhibitory effects of lycopene on carcinogenesis needs further investigations and could involve *(40,41)*: reactive oxygen speces (ROS) scavenging, upregulation of detoxification systems, interference with cell proliferation, induction of gap-junctional communication, inhibition of cell cycle progression, modulation of signal transduction pathways, and effects on the genes governing the androgen stimulation of cell growth, cytokines and on the enzymes producing reactive oxygen species.

Approximately 40 carotenoids are commonly consumed in the US diet and approx 20 can be detected in human serum and tissues *(42)*. Most nutrition research was concentrated

on the six carotenoids found in the highest concentrations in human blood: β-carotene, lycopene, α-carotene, lutein, zeaxanthin, and β-cryptoxanthin. The major dietary lutein sources in the human diet are green vegetables and fruits. Carotenoid consumption and their serum profile vary substantially depending on the origin of the population studied. For example France presents the highest levels of serum lutein and β-carotene and Spain shows the lowest level of β-carotene, along with the highest levels of β-cryptoxanthin *(43)*. American women consume approximately 6 mg of total carotenoids/d *(44)*, the average daily intake of major carotenoids in Spanish population is 3.5 mg/d *(43)* and in Germany total carotenoid intake amounts to 5.33 mg/d with average lutein intake being 1.91 mg/d *(45)*. Daily consumption of lutein and zeaxanthin in American elderly subjects was 2.7 mg for men and 3.09 mg for women *(46)*. In general, the recommended daily intake of carotenoids can only be achieved by consuming 100 to 200 g/d of vegetables and fruits with a particularly high carotenoid content *(47)*.

Low lutein consumption reflects low consumption of fresh vegetable and fruits, changes in nutritional habits, and use of highly processed food. According to National Health Interview Surveys, the intake of lutein declined among different categories of people in the US between 1987 and 1992 *(48)*. There were also significant seasonal differences in plasma carotenoid concentrations in the UK, reflecting a higher intake of lutein during the spring compared with summer and autumn *(49)*. It is interesting to mention that there is also a high positive correlation of lutein ($r = 0.889$) between maternal plasma concentrations and cord plasma *(50)* indicating that the nutritional status of mothers is the major determinant of the lutein status of their babies. In addition it has been shown that breast milk is the major source of lutein to the infants *(51)*. An increased intake of another carotenoid, β-carotene, by lactating women increases the supply of milk β-carotene available to their breast-fed infants.

Carotenoid assimilation from the diet varies significantly depending on many various conditions. However, it seems likely that a substantial proportion of ingested carotenoids could be found in all segments of the digestive tract. Therefore, in combination with other dietary antioxidants carotenoids could promote antioxidant defence in the gastrointestinal tract. Furthermore, carotenoid activities related to the promotion of cell differentiation, regulation of cell proliferation and intracellular communication via gap junctions, as well as regulation of the detoxifying enzymes and enhancement of immune system *(13)*, could also be of great importance in the gastro-intestinal tract.

Carotenoids are known to influence diverse molecular and cellular processes that could be instrumental in their role in reducing the risk for chronic diseases such as CVD and cancer *(52–54)*. Epidemiologic and clinical data showed an inverse association between serum levels of β-carotene and other carotenoids and coronary heart disease (CHD) *(55–57)*. Dietary carotenoids may thus protect against CVD.

The relation between CVD risk and fruit and vegetable consumption was demonstrated by Joshipura et al. *(58)*, who reported a 20% reduction in the incidence of CVD in individuals who consumed the highest quintile of these foods, compared with individuals in the lowest quintile. It is now well established that levels of serum carotenoids may be readily altered by either increasing or decreasing the consumption of fruit and vegetables *(59)*. An increase in serum carotenoids was reported to be accompanied by a significant decrease in serum oxidizability *(59,60)*. Oxidizability in serum can therefore be modified by diet and is related to the carotenoid content of the serum.

In terms of CVD, β-carotene has been the most widely studied because it is one of the most abundant carotenoids *(61)*. Several studies examined the effects of β-carotene in the context of fruit and vegetable intake and also confirmed an inverse association between β-carotene intake and the risk of CVD *(62–64)*. Data from the Rotterdam study on 4802 men and women free of baseline CVD showed that those in the highest tertile of dietary β-carotene intake had a relative CVD risk half of those in the lowest tertile *(65)*.

The mechanisms of action of carotenoids in reducing CVD risk include inhibition of cholesterol synthesis, and an increase in degredation of low-density lipoprotein (LDL) particles through an enhancement of the macrophage LDL receptor activity *(66)*. There is also evidence that carotenoids may reduce the risk of atherosclerosis through inhibition of oxidative damage to LDL; oxidative damage to LDL promotes several key steps in atherogenesis *(67)*. Carotenoids may also reduce the risk of CVD by reducing inflammation as suggested by the inverse association between serum/plasma C-reactive protein (CRP) concentrations and serum/plasma concentrations of β-carotene, α-carotene, and lycopene concentrations *(68,69)*.

As for cancer, several epidemiologic studies also showed that an increased consumption of foods rich in carotenoids is inversely associated with the incidence of major types of cancer in the western world (e.g., carcinoma of the lung, stomach, prostate, mouth, esophagus, colon, or rectum) *(70)*. A similar association was also reported between the concentration of β-carotene in plasma and the risk for cancer *(71)*. Although the biological mechanism for such protection is unknown, various possibilities exist. Carotenoids are potent antioxidants and oxidative stress is known to be involved in carcinogenesis. In a model in vitro system, Bertram and Bortkiewicz showed that carotenoids both with and without provitamin A activity inhibit carcinogen-induced neoplastic transformation. Their results strongly suggest that carotenoids have intrinsic cancer chemo- preventive action in humans *(72)*.

Clinical studies with carotenoid supplementation and some major clinical trials with β-carotene supplementation showed either no or negative effects on CVD and cancer *(73–76)*. Although the reasons for the discrepancy between the results from supplementation and epidemiologic studies are not clear, it has been postulated that supplementation with a single carotenoid at high doses is not sufficient to elicit effects. It has been suggested that a combination of low concentrations of various carotenoids and other micronutrients—as found in fruits and vegetables rather than in individual supplements—appear to be necessary to effect the diverse molecular and cellular processes which form the basis for human health and disease prevention *(77)*. In addition various dietary compounds may provide synergistic effects required for protection against disease *(78)*.

There is no documented evidence that when β-carotene containing natural food sources are consumed in moderation, has negative health implications. Even when very large amounts of carotene rich foods are consumed, the only observed side effect was the occasional appearance of carotenodermia that appears to be harmless and is characterized by yellow or orange tinted skin. The condition disappears spontaneously shortly after the high intake of carotenoid is discontinued. Ingestion of 270 g/d cooked carrots, 180 g/d tomato juice, 300 g/d cooked broccoli or 12 mg/d β-carotene for six wk did not result in the development of carotenodermia *(79)*.

Whereas vitamin A has the potential for acute and chronic toxicity, provitamin A carotenoids do not share the same toxic potential *(80,81)*. As pointed out earlier the

degree of bioconversion of provitamin A in physiological systems is regulated and determined by the vitamin A status of the host *(82–84)*. No adverse effects were reported for any one of the studies in which the value of red palm oil as provitamin A was evaluated *(85–94)*. The duration of these studies varied from two weeks to ten months during which time participants in the study received an estimated 2 to 5 mg of total carotenoids daily.

Data from animal, human and laboratory research suggested that a chronically elevated intake of vitamin A, in the order of 3000 μg/d (about 4 times RDI) may increase the risk of osteoporotic bone disease and fracture, at least in older men and women *(95,96)*. As bioconversion of provitamin A to vitamin A is regulated and determined by the vitamin a status of the host, it could be assumed that the intake of provitamin A carotenoids from fruit, vegetables or red palm oil by older people with an adequate vitamin a status will not pose a risk for the loss of bone mineral density.

β-carotene is the only carotenoid that has been studied extensively in several large-scale primary and secondary prevention trials. Questions as to the safety of the ingestion of high doses of β-carotene have been raised by the α-tocopherol β-carotene cancer prevention study in Finland *(97)*. A statistically significant 18% higher incidence of lung cancer was reported in subjects given 20 mg β-carotene daily compared to subjects receiving a placebo.

Based on a review of published studies the US Preventative Service Task Force does not recommend that people take β-carotene supplements to lower their risk of developing CVD or cancer *(98)*.

3.3. Ascorbic Acid

Vitamin C refers to L-ascorbic acid (AA) and its two-electron reduction product dehydro-L-ascorbic acid. Most animal species synthesize AA from glucose, but human subjects are not able to synthesize it. Therefore AA is an essential dietary component playing an important role in many physiological processes. It is a hydrophilic antioxidant functioning in an aqueous environment and possessing high free-radical-scavenging activity. It can participate in vitamin E recycling thus maintaining efficient antioxidant defence. Fresh green fruits and vegetables are good sources of AA. However, during food processing AA is easily oxidized and as a result AA concentration in such foods is substantially decreased. As a result of its high reducing potential, in combination with iron ions AA can also be a prooxidant. However, it is believed that in physiological conditions, and in the intestinal tract, ascorbic acid performs mainly antioxidant functions. In fact ascorbic acid inhibits chemical synthesis of nitrosamines (animal carcinogens) in the gastric contents and there are suggestions that intakes of ascorbic acid much higher than the RDA may reduce the risk of such diseases as heart disease and cancer *(99)*.

The major advantages of ascorbate as an antioxidant have been described as follows *(100)*:

- Both ascorbate and ascorbyl radical have low reduction potentials and can react with most other biologically relevant radicals and oxidants.
- Ascorbyl radical has a low reactivity as a result of resonance stabilisation of unpaired electron and readily dismutates to ascorbate and dehydroascorbic acid (DAA).

- Ascorbyl radical and DAA can be converted into active ascorbate form by enzyme-dependent or independent pathways. In particular, the ascorbyl radical can be reduced by NADH-dependent semidehydroascorbate reductase or by thioredoxin reductase. At the same time DAA can be reduced to AA by GSH, lipoic acid or glutaredoxin.

The current recommended dietary allowance (RDA) of vitamin C is 75 mg for women and 90 mg for men, based on the vitamin's role as an antioxidant as well as protection from deficiency *(101)*. Recently reviewed data have suggested that an intake of 90 to 100 mg vitamin C/d is required for optimum reduction of chronic disease risk in nonsmoking men and women suggesting a new RDA of 120 mg vitamin C/d *(100,102)*. Therefore, it was suggested that five servings of fruits and vegetables/d may be beneficial in preventing cancer and providing sufficient vitamin C intake for healthy people.

High intakes of the vitamin are generally well tolerated, however, a Tolerable Upper Level (TUL) was recently set at 2 g based on gastrointestinal upset that sometimes accompanies excessive dosages. Indeed, the most common adverse effects of high vitamin C intakes (>2 g/d) are gastrointestinal symptoms such as nausea, abdominal cramps, and diarrhoea *(99)*. After exclusion of the vitamin supplements the symptoms usually disappear within a week or two with no further consequences. Several populations warrant special attention with respect to vitamin C requirements. These include patients with periodontal disease, smokers, pregnant and lactating women, and the elderly *(101)*.

3.4. Flavonoids

Flavonoids are low-molecular-weight polyphenolic substances based on the flavan nucleus. They are widespread in nature, occurring in all plant families, and are found in considerable quantities in fruits, vegetables, grains, cola, tea, coffee, cocoa, beer, and red wine *(103)*. The list of known flavonoids substantially increased from more than 4000 in 1996 *(104)* to over 8000 individual compounds known in 2000 *(105)*. The major flavonoid classes include flavonols, flavones, flavanones, flavanols (catechins), anthocyanidins, isoflavones, dihidroflavonols, and chalcones *(104)*. Representatives of major groups of flavonols were characterized as having antioxidant properties in vitro and in vivo *(105)*.

These compounds have received substantial attention in recent years. The major driving forces of research in the field were the positive effects of fruits and vegetables on human health and their preventive role in the development of various diseases, especially cancers. The flavonoid content in fruits and vegetables can be as high as 300 mg/kg fresh weight *(106)*. In fact, alleged health-promoting effects of flavonoids are usually attributed to their powerful antioxidant activities, but evidence for in vivo antioxidant effects of flavonoids is confusing and equivocal *(107)*. The major problem with antioxidant properties of these compounds is their low availability from the dietary sources. For example, in human blood or urine polyphenol concentrations was shown to be in a range 1 to 2 μM *(108,109)* in comparison with the general concentration of antioxidants in human plasma to be about 1000 μM *(110)*.

Therefore, it has been suggested that the digestive tract is the major site of antioxidant defence afforded by polyphenolic compounds such as flavonoids *(111–113)*. Indeed, phenols might exert direct effects within the gastrointestinal tract, because of the high concentrations present. These effects could include binding of prooxidant iron,

scavenging of reactive nitrogen, chlorine, and oxygen species, and perhaps inhibition of cyclooxygenases and lipoxygenases *(107)*.

Daily intake of flavonoids varies substantially between different countries and is highest in Asian population and in vegetarians. In particular, average daily intake of flavonols in Asian countries comprised about 68 mg and isoflavones 20 to 240 mg *(103)*. In contrast, the mean intake of flavonols of the German population was about 11.5 mg, mainly derived from fruits and vegetables, but also from black tea and red wine *(114)*. Indeed, naturally occurring polyphenolic compounds may play a role in the protective effects of fruits and vegetables against cancers in general, and they appear to have considerable potential as chemopreventive agents against neoplastic changes in the alimentary tract *(115)*. In general flavonoids can prevent LDL oxidative modification by scavenging ROS, chelating transition metal ions, or inhibiting lipoxygenase and this leads to the prevention of atherosclerosis. For example, a number of studies have shown that consumption of soy is antiatherogenic and that the isoflavones genistein, diadzein, and biochanin, which inhibit lipoprotein oxidation in vitro and suppress formation of plasma lipid oxidation products in vivo, are most likely responsible for this effect *(116)*.

However, there are no data available on the long-term effects of flavonoid dietary supplementation on humans. A serious problem with flavonoids is that, depending on conditions, they could be antioxidants or prooxidants, antimutagens or promutagens. Therefore unregulated use of flavonoid-containing supplements can have a detrimental effect on human health. For example, the results obtained by Silva et al. *(117)* suggest that there is a range of flavonols whose genotoxicity in eukaryotic cells depends on their autooxidation. These flavonols can autooxidize when the pH value is slightly alkaline, such as in the intestine, and therefore can induce genotoxicity in humans. Clearly more research is needed to clarify health benefit and potential dangers of these compounds.

Comparatively low bioavailability and antioxidant potential of various flavonoids could be beneficial for the human providing antioxidant protection in various part of the digestive tract, including the large intestine where levels of other antioxidants would be quite low.

3.5. Other Polyphenolics

Cereal brans contain significant quantities of the phenolic ferulic acid and diferulic acid and their potential health benefits (protection of LDL from oxidative modification and reduction in atherogenesis as well as inhibitory effects on tumor promotion and chemopreventive properties) have been related mostly to their antioxidant activity *(118)*.

3.6. Spices and Essential Oils

Addition of spices to food is a common procedure in most cultures. The seasonings contribute a pleasant flavor and recently it has been shown that they contain a range of antioxidant compounds and it seems likely that only a small proportion of them have been isolated and identified *(119)*. They include such phenolic diterpenes as carnosoic acid, carnosol, rosmaridiphenol, and rosmariquinone from rosemary, sage, and summer savory. In other spices a range of flavonoids have been identified. In general, spices and herbs have been shown to have over 100 compounds with high antioxidant activity including 26 active compounds from the *Labiatae* family, *Rosmarinus officinalis*, *Thymus*

vulgaris, *Origanum vulgare* and *O. majorana*, over 40 antioxidative compounds from *Zingiber officinale* and 26 compounds from *Curcuma domestica (120)*. Spices are effective in prevention food deterioration during storage and this explains why traditional diets in countries with high temperature (e.g., India, Thailand, Mexico) are usually rich in spices.Essential oils from aromatic and medicinal plants have been shown to have antibacterial, antimycotic, and antioxidant properties. Recently the essential oils from black pepper, clove, geranium, melissa, nutmeg, oregano, and thyme and 33 phytoconstituents have been assessed in vitro *(121)*. All the compounds demonstrated antioxidant capacities superior to the water-soluble α-tocopherol analogue Trolox with the exception of the essential oil melissa and three phytoconstituents. The best results were obtained with clove, oregano, and thyme oils and their corresponding phytoconstituents namely eugenol, carvacrol, and thymol. Again in the GIT antioxidant properties of various compounds from spices and herbs would contribute to total antioxidant potential.

4. SYNTHETIC ANTIOXIDANTS

Antioxidants in foods may be endogenous origin or may be added externally to preserve their lipid components from peroxidation. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) are commonly used in food formulations. However, because of safety concerns, public interest shifted from synthetic to natural antioxidants. As a result mixed tocopherols, herbal extracts such as those of rosemary and sage, as well as tea extracts have been commercialized for food and nutraceutical applications *(122)*.

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26 Chia Seeds and the Columbus Concept
Bakery and Animal Products

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Abstract

Cardiovascular disease (CVD) is a major problem worldwide, and is the most common cause of death in the Western world. Diets high in total fat, saturated fatty acids, trans fatty acids and having high ω-6:ω-3 fatty acid ratios have been directly linked to risk of suffering coronary heart disease (CHD). Chia (*Salvia hispanica* L) is the highest known plant source of ω-3 fatty acids, and also is a good source of fiber and protein. Its natural antioxidants keep it stable, even when ground, so it can be stored for extended periods of time without degradation taking place. Chia has been fed to chickens and other animals, and has significantly improved the lipidic profile of the products produced as well as the serum of rats, with no evidence of a decrease in product quality or animal health. This is unlike flaxseed and fish oil/meal which have been shown to give off flavors (fishy flavor) to eggs, and in the case of flaxseed, reduced egg production. Adding chia to bread and other bakery products could provide consumers with an easily obtainable, low cost food that would significantly increase their ω-3 intake without having to change their normal diet, or be faced with an allergic reaction which can occur when eating fish. As chia becomes more of a mainstream source of ω-3 fatty acids its price will decrease, and if added to bakery products, it would provide a large percentage of the world's population with a low cost source of ω-3 fatty acids.

Key Words: Chia; ω-3 fatty acids; flax; fish; bread.

1. INTRODUCTION

Cardiovascular disease (CVD) is the main cause of death worldwide. At the beginning of the 21st century CVD accounted for only 10 to 30% of all deaths and was primarily a problem in developed countries. Today it is a growing concern in developing countries as well, and has become the main cause of death in many of them *(1,2)*.

Since 1900 CVD has been the number one killer in the United States (US) every year but 1918. The main form of CVD is coronary heart disease (CHD) and it comprises more than half of all CVD events in men and women under age 75 *(3)*. In fact, CHD is the most common cause of death in the Western world. In the US and the European Union (EU) it accounts for over 600,000 deaths annually *(4)*. CHD has major economic consequences. Estimated direct and indirect costs of CHD are 132.2 and 5.6 billion dollars/yr for the US and UK, respectively *(4,5)*.

It is estimated that up to 30% of CHD deaths result from unhealthy diets *(5)*. Diets high in total fat, saturated fatty acids, trans fatty acids, and having high ω-6:ω-3 fatty

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acid ratios have been directly related to risk of suffering CHD. On the other hand, ω-3 fatty acid enriched diets are inversely linked with risk of CHD *(6–9)*. The problem is that western diets are typically low in ω-3 fatty acids, and high in saturated and ω-6 fatty acids *(10)*.

At the end of last century health organizations from many countries began recommending increased ω-3 fatty acid intakes *(11–14)*. However, as a result of agricultural changes over the last 100 yr food production became reliant on only a few species, all of which are rich in ω-6 fatty acids and low in ω-3 fatty acids. These species are also used for feeding domestic animals, producing animal products having the same lipidic characteristics as the food they eat *(15,16)*. This situation has heightened the need to identify and put into commercial production species rich in ω-3 fatty acids which could be used as food for humans and domestic animals, thereby providing consumers with food lipidic profiles similar to those under which the human genome evolved *(17)*.

One program which was initiated to identify and put into commercial production new crops, and in particular new sources of ω-3 fatty acids, was the Northwestern Argentina Regional Project. This began in 1991, with a goal being to add chia (*Salvia hispanica L.*) as a crop to modern agricultural production practices *(18)*.

Today chia is making a comeback and is joining a small group of commercially available ω-3 fatty acid sources which can be used as a food for both humans and animals. Other foods included in this group are flaxseed, fish, and algae. Of these, chia and flax are sources of α-linolenic (ALA = 18:3) fatty acid. Fish and algae are sources of what are called long chain ω-3 fatty acids, that is eicosahexaenoic (EPA = 20:5) and docosahexaenoic (DHA = 22:6) fatty acids.

2. CHIA (*S. hispanica* L)

As early as 2000 BCE, chia was one of the main foods of Mesoamerican nations, including the Aztecs and Mayans, and along with maize, beans, and amaranth were core elements of their diets *(19–22)*. Its importance in the daily life of these cultures precipitated a rapid decline in its use, brought about because of persecutions and prejudices of the European conquerors. It went from being one of the main foods of the 16th century, to having almost disappeared by the middle of the 20th century. It survived only because a few small groups of individuals retained some of the customs of ancient Americans *(23)*.

3. ω-3 SOURCES: CHARACTERISTICS AND COMPARISONS

From a nutritional point of view, both for animals and humans, chia, flaxseed, fish and algae have very different characteristics (Table 1). Fish and chia have a long history of use in the human diet, with fish being a staple food for many populations along oceanic coasts. Although the use of this resource is declining *(24–26)*, it is still a basic dietary component in many regions.

Flax and marine algae have never been considered important nutritional sources in the history of mankind. Additionally, flax has been questioned as a food source since it contains a number of components that interfere in the normal development of humans and animals *(27–39)*. None of the toxic factors found in flax have been found in chia

Table 1
Characteristics of Four Sources of ω-3 Fatty Acids

ω-3 source	*Fish oil (157)**	*Algae (146)***	*Flaxseed (158)*	*Chia (159)*
Origin	animal	vegetal	vegetal	vegetal
History as human food	no	no	no	yes
Primarily used to manufacture	feed	feed	industrial	food
ω-3 fatty acid (FA)	EPA and DHA	DHA	ALA	ALA
Total ω-3 fatty acids FA (%)	30	37	58	64
Total saturated FA (%)	27	50	7	9
Cholesterol	yes	no	no	no
Antinutritional/toxic factors	yes	no	yes	no
Fat stability	very low	very low	low	high
Natural antioxidants	no	very low	very low	high
Antioxidant needs	yes	yes	yes	no
Off-flavor (fishy flavor)	yes	yes	yes	no
Handling & storage	difficult	difficult	difficult	easy

*Menhaden oil.
**DHA Gold™ *(Schizochytrium sp.)*.
Adapted with permission from ref. *156*.

(40–48). In reality the main use of flax is in the manufacture of industrial products such as coatings, floor coverings, paints and varnish.

Terrestrial (plant) ω-3 sources have an important advantage over algae and fish from a health standpoint because they contain significantly less saturated fatty acids (e.g., myristic, palmitic, and stearic). Chia oil has 2.8 and 5.1 times less saturated fatty acids than does menhaden and algae oil, respectively *(23)*. Dietary saturated fatty acids are independent risk factors associated with CHD, with their negative effects on blood low-density lipoprotein cholesterol (LDL) being greater than those of dietary cholesterol *(49)*. Stearic fatty acid is considered much less hypercholesterolemic than palmitic and myristic fatty acids *(50,51)*, or not hypercholesterolemic at all *(52,53)*, and hence is of a lesser concern. If one considers the other two fatty acids it can be seen that chia has 3.3 and 7.1 times less myristic and palmitic fatty acid than do menhaden and algae oil, respectively *(23)*. Therefore chia is much healthier than these other two sources of ω-3 fatty acids.

Unlike flaxseed, fish oil, and algae, consumption of chia is not regulated. The Food and Drug Administration (FDA) stated "chia (*S. hispanica*) is considered a food and hence is exempt from regulations ... chia has been consumed by native cultures for long periods of time, and we are not aware of any safety concerns *(54)*." A number of studies have verified the safety of chia because animal food products have been effectively enriched with ω-3 fatty acids using chia seed as a feed, without any negative effects on animal development being recorded. Some products that have been produced using chia are: eggs, poultry meat and milk as shown in Table 2 *(40,41,55–59)*.

Chia has a very low sodium content *(23)*. Chia seed has 37, 39, 49, 57, 65, 68% less sodium than flaxseed, algae *(Schizochytrium sp.)*, tuna *(Euthynnus pelamis L.)*, wild

Table 2
ω-3 Enriched Foods Produced by Adding Chia Seeds

Food	*Chia added to the ration* %	*ω-3 content with chia and without chia mg/100 g of edible portion*	*ω-6*	*ω-6:ω-3*	*DV of ω-3 with chia*	
					Ratio	*%*
Eggs						
white	14	1193	84	1988	1.7:1	92
brown	14	1134	81	1892	1.7:1	92
Poultry meat						
white	10	695	84	806	1.2:1	54
dark	10	611	110	1973	3.2:1	47
Cow's milk	2	45	34	283	6.3:1	8.5
Wheat bread	10	4030	0.6	2210	1:1.8	310

Adapted with permission from refs. *23,57,137,* and *156*.
Note: Percentage of daily value (DV) per serving are based on recommendations for 2000 calorie diet.

salmon, halibut *(Hippoglossus hippoglossus L.)*, and bluefish *(Pomatomus saltatrix L.)*, respectively *(60–62)*. This is significant in that nutritional studies have shown a positive relationship between sodium intake and high blood pressure. For people with high blood pressure, chia has a distinct advantage compared with other sources of ω-3 fatty acids *(63)*.

3.1. Flaxseed

Human consumption of flaxseed oil has been banned in France since 1973, and is restricted in Germany, Switzerland and Belgium *(64–66)*. In the US, human consumption is not prohibited, however flaxseed has not received GRAS status from the FDA. This means that should a company decide to include flax in a food product, it shall be liable for the safety of that product *(67,68)*.

Restrictions on human use of flaxseed as a food mainly result from the presence of toxic cianogenic compounds (e.g., linamarin, linustatin, and neolinustatin) and the vitamin B6 antagonist factors it contains *(69–74)*. Recent findings show that low levels of B vitamins in the blood are linked with an increased risk of fatal coronary heart disease and stroke *(75)*. Homocysteine, a nonprotein forming sulfur amino acid, is not a normal dietary constituent and is elevated when folic acid and B vitamin levels are inadequate *(76–78)*. Researchers believe that when body cells dump too much homocysteine into the blood, artery linings become irritated, encouraging the formation of plaque deposits that cling to artery walls *(79)*. An elevated level of serum homocysteine concentration is now recognized as an important, independent risk factor for cardiovascular disease and stroke *(80,81)*.

Recent research on animals has shown the negative effects eating flax has on pregnancy and reproductive development. These effects have been attributed to the action of the compound diclycoside ecoisolariciresinol (SDG) which through microbial action acts as estrogenic depressor or potentiator in mammals. Flax is the richest source of

SDG known, and therefore special caution is recommended if it is consumed during pregnancy and lactation *(82,83)*. The Government of Canada, through Health Canada (2004), recommends avoiding the use of flax if you are pregnant or breast-feeding *(84)*.

Owing to the general availability of flax (used primarily as an industrial oil), and because of its relatively low price, there have been many attempts to use it as an ω-3 fatty acid source in animal production. Numerous publications have shown the negative effects that the anti-nutritional factors in flax have on the development of layers, broilers, beef cattle, and rabbits *(27,29–32,34,36–39,55,85)*. Thus in order to use flax the seed must be detoxified using solvents, and even then the seed cannot be completely detoxified *(86,87)*.

3.2. Fish and Fish Products

A major limitation of fish as a food is that it has been recognized as a potent allergen. Reactions to fish are among the most commonly encountered food allergies in children and adults *(88–91)*. Allergic reactions are now a leading cause of illness and death, particularly in young children, and an increase in the prevalence of allergic disorders has been documented in several developed countries *(92–94)*. To protect individuals from such reactions, effective January 1, 2006 the FDA (2006) is requiring that food labels list the amount of any ingredient that contains protein derived from the eight major allergenic foods, this includes fish and crustacean shellfish *(95)*.

Restrictions on consumption of fish oil as a food exist. For example consider the oil produced from the species known as menhaden *(Brevoortia tyrannus)*. When the FDA (2005) gave this oil a generally recognized as safe (GRAS) status for use as a food ingredient it came with limits on the maximum levels that should be consumed *(96)*. These levels become even more restrictive when menhaden oil is consumed in combination with other oils that are significant sources of EPA and DHA. FDA (2005) concluded "that these limits are necessary to ensure that daily intakes of EPA and DHA do not exceed 3.0 g/person/d *(96)*."

The maximum daily intake of EPA and DHA has been set as a safeguard against possible adverse effects these fatty acids can have on bleeding, glycemic control in noninsulin dependent diabetics, and low-density lipoprotein (LDL) cholesterol levels. The maximum amount of menhaden oil that can be used in different products as set by the FDA (2005) is 5% in baked goods and baking mixes, milk products, meat products, gravies, sauces and snack foods; 4% in cereals; 3% in soup mixes and poultry products; 2% in pasta; 1% in puddings and gelatins, processed fruits and vegetable juices; and 0.5% in nonalcoholic beverages *(96)*.

Another important consideration is that fish oils are animal products, hence they contain cholesterol, with the amount varying by species. For instance cholesterol content/100 g of oil derived from herring (*Clupea harengus*) is 766 mg, sardine (*Clupea harengus harengus*) is 710 mg, cod liver (*Gadus morrhua*) is 570 mg, menhaden is 521 mg, and salmon (*Salmo salar*) is 485 mg *(60)*. Chia, flax and algae do not contain cholesterol as they are not from the animal kingdom.

Because fish oils are generally by-products obtained during preparation of fish meal their composition is not uniform and changes according to the source of the marine oil and degree of hydrogenation that takes place during processing. The range in fatty acid composition that occurs because of season, location, species, etc. is well

known, and wide variations in commercial fish oils/meal have been reported *(97–99)*. As examples menhaden and salmon oil have approximately equivalent Environmental Protection Agency (EPA) levels (13%), whereas sardine and cod liver oil have about 6.5% EPA *(60)*.

Another consideration is that oils derived from fish livers, such as cod liver oil, have higher vitamin A levels than do whole-body fish oils. Increased dietary vitamin A has been shown to antagonize (interfere with) vitamin E status in poultry and other animals *(100–102)*.

World fish stocks are in decline through over fishing and pollution of waterways. Today, high concentrations of toxic substances such as organic pollutants (Σ14 PCB, DDT, oxychlordane, and others) and mercury in marine fish are a cause of concern. Recent studies in Canada found PCB pollutant levels in the blood of maternal women to be greater than the recommended values for women of reproductive age *(103,104)*. Similar findings were reported in Sweden by Svensson et al. (1991) in populations consuming large amounts of fish *(105)*. These individuals had significantly higher levels of dioxin in their body fat than nonconsumers.

Mercury contamination in fish is a health concern, since almost everyone who consumes fish has at least trace quantities of methylmercury in their body *(106)*. Inuits residing in Nunavik (Arctic Québec, Canada) regularly consume large quantities of seafood. Studies have shown that a significant proportion of their reproductive-age women have mercury concentrations exceeding those recommended. These individuals have been associated with subtle neurodevelopmental deficits *(107)*.

Recently the FDA advised pregnant women, women of childbearing age who may become pregnant, nursing mothers and young children on the hazards of consuming fish that may contain high levels of methyl mercury. The FDA is advising this segment of the population not to eat shark, swordfish, king mackerel, and tilefish *(108,109)*. Similarly in the UK, the Food Standards Agency advised the same group of women as well as children under 16 to avoid eating shark (*Squaliformes*), marlin (*Makaira nigricans/Tetrapterus albidus*) and swordfish (*Xiphias gladius*). It also advised that at a maximum, no more than one portion of these fish should be consumed weekly by other adults *(110)*.

Aquiculture has not brought a solution to the problem of contaminated fish. An increasing number of studies show a high degree of contamination in farmed fish, and in some cases this is even higher than in wild fish *(111,112)*. Hites et al. (2004) analyzed wild salmon from around the world along with farmed salmon from Norway, Chile, Scotland, British Columbia, Eastern Canada, the Faroe Islands, Maine, and Washington, because these represent the top salmon farming regions in the world *(113)*. They stated, "frequent consumption of farmed salmon and wild Pacific Chinook salmon will increase human dietary exposure to polybrominated diphenyl ethers (PBDEs), much more so than will consumption of most other wild Pacific salmon."

Until a few years ago fish products from marine origin were the main source of ω-3 fatty acid, not only for human consumption but as a feedstock for farmed fish as well. Because of over fishing and hence declining marine fish meal/oil availability and increased prices, commodity grains have been used more and more as food for aquiculture. As the nutritional value and safety of fish and fish products produced depends on what they have been fed, farmed fish fed grains low in ω-3 fatty acids are low in these fatty acids as well. As an example Seierstad et al. (2005) reported that consumption

of fillets from salmon fed marine diets lowered the risk of suffering CHD, whereas people consuming fillets from salmon fed rapeseed oil did not *(114)*.

With confirmation that consuming fish having a low ω-3 fatty acid content does not produce favorable effects in patients with CHD, and knowing that not all fish can elongate and desaturate ω-3 fatty acid to produce EPA and DHA, means fish need to be fed these fatty acids derived from marine fish meal/oil if they are to contain them. Considering that 3 kg of fish products must be fed to produce 2 kg of fish, questions about the sustainability of fish farms as an ω-3 fatty acid source arise *(115,116)*.

As a result of readily available supplies of fish oils and meals they have been used extensively as ω-3 sources in poultry *(117–122)*, cow *123–125)*, pig *(126)*, and beef cattle *(127)* diets to produce healthier foods. There have been problems, however. For example reproductively active hens exhibited increased hepatic lipidosis following 6 mo of feeding 3% menhaden fish oil. Van Elswyk et al. (1994) suggested that this came about because the oil enhanced the lipogenic activity of the liver in the hens *(128)*. This exposes another problem with fish oil and meal.

3.3. Algae

Traditionally algae have not been a part of human or animal diets (with the exception of fish). The need to use sodium chloride (NaCl) to produce the artificial environment in which algae are grown, combined with the use of solvents for oil extraction, raises environmental concerns about this product *(129,130)*.

Oil from one species of algae (*Schizochytrium sp.*) has received GRAS status from the FDA (2004), but this applies only when daily intake of DHA does not exceed 1.5 g *(131)*. The FDA also placed limits on the maximum amount which can be used in various categories of food. The limit for meat products, milk products, cookies and crackers, snack foods, cereals, backed goods and backing mixes, and breads and rolls is: 2.9,1.45, 1.45, 1.45, 1.16, 1.16, 0.29%, respectively *(131)*.

4. ω-3 ENRICHED FOODS: BAKERY PRODUCTS AS AN ALTERNATIVE

The market for functional foods, including products enriched with ω-3 fatty acids, has reached a significant level and is expected to grow in several countries following increased consumer interest in the nutritional content of food *(132)*. Most shoppers believe food can offer benefits that reach beyond basic nutrition, and can provide functional nutrition for disease prevention and health enhancement. The first consideration, however, when making healthy choices is taste. Today's consumers are less willing than ever to compromise taste, for health benefits *(133)*. A survey conducted for the United Soybean Board (2005) in the US showed that 74% of consumers had changed their eating habits because of nutrition or health concerns *(134)*. The survey also reported that 48% of US consumers recognize ω-3 fatty acids as being healthy.

In general ω-3 fatty acid enriched products are more expensive than comparative products having a low ω-3 fatty acid content. Cost is a major problem for low-income people who want to consume ω-3 fatty acid enriched products. An informal survey comparing supermarket prices of products in selected countries of the US, EU, and Africa showed 17 to 175% and 13 to 112% higher prices for ω-3 enriched eggs and dairy milk, respectively, compared with common products *(23)*.

Bread is a typical component of Western diets, and many other countries as well. Archeology and history show that it has been used as food for humans for a significant period of time. Cereals were ground in prehistoric Britain to make a bran-like grain, then fashioned into flat cakes and cooked. In Egypt, where civilization was considerably more advanced, bread was a regular part of daily life, as it was in the Greek and Roman civilizations *(135)*. Although bread consumption declined after World War II, before which it was the single most important food in the European diet, it still is consumed, in large quantities, by many people in Europe and other Western countries. In developed countries bread consumption, particularly for those prepared with whole grain flour and multi-grain flour, has increased as a result of an increase in the nutritionally conscious proportion of the population *(136)*. An advantage of making ω-3 fatty acid enriched bread and bakery products available is that they could be served alongside other foods, without having to change normal meal customs.

Chia provides the same advantages over flaxseed when used to make ω-3 enriched bread, as when used as animal feed *(41,55,58)*. A trial which compared chia and flaxseed for making ω-3 fatty acid enriched bread showed the chia bread to have a significantly higher (294%) ω-3 fatty acid content (Table 3), and better acceptability than did either the flax or control breads *(137)*. Considering the ALA content of flaxseed and chia, and the incorporation of it into the enriched breads which took place, chia had a much higher conversion efficiency, almost 368% greater, than flax. Similarly animals fed chia enriched diets have shown higher ALA and total ω-3 fatty acid deposition than animals fed flaxseed. As examples, eggs from hens and white meat from broilers fed chia have 250% and 125% higher ω-3 fatty acid contents, respectively, than those products produced when flaxseed is fed *(41,55)*.

The difference in deposition could be related to the different antioxidant compounds found in flaxseed and chia. Chia seed extracts have been shown to have strong antioxidant activity *(138)*. The most important antioxidants are chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol flavonols *(138,139)*. Caffeic acid and chlorogenic acid have been shown to have significantly stronger antioxidant properties than a number of other common antioxidants such as the tocopherol compounds which are the main antioxidants found in flaxseed *(60,140)*. In the case of bread they appear to prevent not only degradation of ALA, but also a decrease in total lipid.

Bautista-Justo et al. (2005) found superior antioxidant capacity in chia enriched bread, compared with flaxseed enriched bread *(137)*. The trial also showed that although the enriched breads have almost the same ω-6:ω-3 ratios, the chia bread had three times more ω-3, 2.9 times more EFA, and 1.6 times lower SAT:ω-3 and SAT:EFA ratios than the flaxseed enriched bread (Table 3) *(137)*.

Quantity and quality of dietary fiber in chia gives it another nutritional advantage for bakery products. Chia has 28% more total dietary fiber than flax *(61)*, and chia fiber has five times the water retention capacity of flaxseed *(141)*. Consumption of foods high in dietary fiber, both total and soluble, has reduced serum cholesterol. Significantly ($p < 0.05$) greater decreases in total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were found in the serum of rats fed chia seed, compared with rats fed chia oil. This suggests that a soluble fiber effect with chia seed, but not with chia oil may exist *(142)*.

Table 3
Fatty Acid Composition of ω-3 Enriched Breads Produced by Adding Chia or Flax Seeds

	Control	*Chia 10%*	*Flaxseed 10%*
Fatty acid	*mg/100 g of edible portion*		
14:0	0.02	0.04	0.03
16:0	0.26	1.12	0.58
16:1	0.01	0.02	0.01
18:0	0.12	0.41	0.26
18:1	0.45	2.67	1.37
18:2	0.30	2.21	0.79
18:3	0.05	4.03	1.37
SAT	0.4	1.57	0.87
EFA	0.35	6.24	2.16
ω6:ω-3	6:1	1:1.8	1:1.7
SAT:EFA	1.4:1	1:4.0	1:2.5
SAT:ω-3	8:1	1:2.6	1:1.6

Adapted with permission from ref. *137*.

5. CONCLUSIONS

Chia seed is an excellent alternative to flaxseed and marine sources when used for the production of foods having a high ω-3 content. There is scientific evidence that ω-3 enriched foods from animals fed flax seed/oil, fish oil/meal and algae, or foods that have these components artificially added, tend to have off-flavors *(120,122,126,143–152)*. Quite the opposite, chia has been shown to produce foods lacking off-flavors *(40,41,43,57)*. As consumers are concerned about ω-3 enriched foods smelling or tasting like fish *(121,153)*, the absence of atypical organoleptic characteristics represents a significant commercial advantage for chia.

Food off-flavors are often associated with lipid oxidation *(154)*. In a number of cases inclusion of antioxidants in products produced using flaxseed and fish oil resulted in a significant improvement in oxidative stability *(155)*. This would tend to indicate that the difference in organoleptic characteristics of foods produced by adding chia rather than other ω-3 sources can be attributed to the powerful action of the antioxidants it contains, as has been suggested in a number of papers *(41,55,57,137,156)*.

Increasing the diversity of ω-3 fatty acid enriched foods on the market, and making them available at a low cost, could increase the intake of these fatty acids. This would contribute to better control of CHD, not only in developed countries, but in undeveloped countries as well. The results would be decreased costs not only in terms of saving lives, but in reducing the amount of resources needed to combat such diseases.

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27 Health Effects of Foods Rich in Polyphenols

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Abstract

We have reviewed literature regarding the health benefits of foods rich in phenolic compounds. Numerous epidemiological studies indicate an inverse association between fruit and vegetable intake and the risk for cardiovascular disease (CVD), ischemic stroke, and other chronic diseases. Besides providing essential vitamins, minerals, and dietary fiber, fruits contain polyphenols that exhibit antioxidant, anti-inflammatory, and lipid lowering properties. Several types of berries, cherries, black grapes, and tea are rich sources of dietary phenolic compounds. There are a number of in vitro studies that demonstrate that these compounds reduce oxidative stress and inflammatory response. Data from human feeding studies are limited and inconsistent. Regardless of the inconsistencies, results from human intervention studies do show reduction in oxidative stress, markers of inflammation, serum triglycerides and low-density lipoprotein-cholesterol when the diets were supplemented with fruits or fruit extracts rich in polyphenols. The inconsistencies may be due to the differences in the amount and type of the polyphenols consumed, duration of supplementation, basal diet and health status of the subjects.

Key Words: Polyphenols; antioxidants; endothelial function; inflammation; blood lipids; cardiovascular disease (CVD).

1. INTRODUCTION

The link between diet and health has been recognized since ancient times. Physicians have treated their patients with herbs and foods believed to have medicinal properties *(1)*.

Epidemiological studies have shown association between the consumption of diets rich in fruits and vegetables and a lower risk of chronic diseases like cancer *(1–3)*, heart disease *(4,5)*, and stroke *(6,7)*. Reduced risk of obesity and better control of diabetes are some additional benefits that are likely to follow from increased consumption of plant foods *(1)*. Fruits and vegetables, apart from being good sources of vitamins, minerals, and fiber, are also rich sources of potentially bioactive compounds known as phytochemicals. Phytochemicals are not designated as nutrients, but much of the disease prevention potential of fruits and vegetables in human health is thought to be provided by those compounds. Polyphenols are one of the most extensively studied phytochemicals. The purpose of this chapter is to provide a brief overview of the various types of phenolic compounds, their dietary sources, and their role in prevention and treatment of chronic diseases.

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2. TYPES OF PHENOLIC COMPOUNDS

Based on the number of phenol rings and structural elements that bind these rings to one another, phenolic compounds are classified as phenolic acids (single rings), flavonoids, stilbenes, and lignans (multiple rings). Chemical structures of the different phenolic compounds can be found in recent reviews *(8–10)*. Common dietary sources of polyphenols are given in Table 1. These compounds are nonnutritive, secondary products of plant metabolism and serve functions essential for growth and survival, like protection from ultraviolet (UV) radiation as well as defense against pathogens and predators. They also provide color and flavor to the plant. Several hundred different polyphenols have been identified in various fruits, vegetables and other edible plants.

2.1. Phenolic Acids

Phenolic acids are derived in most plants from the amino acid phenylalanine and account for about one third of our total phenolic compound intake *(11)*. Two broad classes of phenolic acids have been recognized; derivatives of benzoic acid and derivatives of cinnamic acid. Hydroxybenzoic acid content of edible plants is generally very low with the exception of red fruits, black radish and onions, which contain up to several tens of milligrams/kg fresh weight *(12)*. Tea is an important source of a type of hyroxybenzoic acid called gallic acid and contains up to 4.5 g/kg fresh weight *(12)*.

Hydroxycinnamic acids are more common and consist chiefly of *p*-coumaric, caffeic, ferulic acid, and sinapic acid. Hydroxycinnamic acids are abundant in fruits like blueberries, kiwis, plums, cherries, and apples; having between 0.5 and 2 g hydroxycinnamic acid/kg fresh weight *(13)*. Caffeic and qunic acid combine to form chlorogenic acid, which is present in high concentrations in coffee; a single cup may contain 70 to 350 mg chlorogenic acid *(14)*.

2.2. Flavonoids

Flavonoids are more common polyphenolic structures in plants and are responsible for the color of the fall foliage and flowers. Based on their chemical structure, flavonoids are classified into anthocyanidins, flavanols, flavonols, isoflavones, and flavonones. Common dietary sources of flavonoids are given in Table 1.

2.2.1. ANTHOCYANIDINS

Anthocyanidins are water soluble pigments present in the vascular sap of the epidermal tissues and impart pink, red, blue or purple color to flowers, fruits, and vegetables. In human diets, anthocyanins are most abundant in fruits such as blackcurrants and blackberries in which the anthocyanin content reaches up to 2 to 4 g/kg fresh weight depending on the color intensity *(8)*. Anthocyanins are often found in the skin of the fruits and vegetables except for some red fruits like cherries and strawberries in which they are present also in the flesh *(8)*. Anthocyannins are also present in some cereals, and vegetables like cabbage, beans, onions and radish. Wine is also an important source of anthocyanins, containing about 200 to 350 mg/L and these are transformed into complex structures as the wine ages *(15)*.

Table 1
Common Dietary Sources of Polyphenols

Polyphenols	*Source*	*Serving size*	*Polyphenol content mg/serving*
Hydroxybenzoic acid *(12,13)*	Blackberry	100 g	8–27
Protocatechuic acid	Raspberry	100 g	6–10
Gallic acid	Black currant	100 g	4–13
	Strawberry	200 g	4–18
Hydroxycinnamic acids	Blueberry	100 g	200–220
(12,13,14,152)	Kiwi	100 g	60–100
Caffeic acid	Cherry	200 g	36–230
Chlorogenic acid	Plum	200 g	20–230
Coumaric acid	Aubergine	200 g	120–132
Ferulic acid	Apple	200 g	10–120
Sinapic acid	Cider	200 mL	2–100
	Coffee	200 mL	70–350
Anthocyanins *(15,153,154)*	Aubergine	200 g	1500
Cyanidin	Blackberry	100 g	100–400
Pelargonidin	Black Current	100 g	130–400
Malvidin	Blueberry	100 g	25–500
Peonidin	Black grape	200 g	60–1500
Delphinidin	Cherry	200 g	70–900
	Rhubarb	100 g	200
	Strawberry	200 g	30–150
	Red wine	100 mL	20–35
Flavonols *(25,155–159)*	Yellow onion	100 g	35–120
Quercetin	Curly Kale	200 g	60–120
Kaempferol	Leek	200 g	6–45
Myricetin	Broccoli	200 g	8–20
	Blueberry	100 g	3–16
Flavanones *(160–162)*	Orange juice	200 mL	40–140
Hesperetin	Grapefruit juice	200 mL	20–130
Naringenin	Lemon juice	200 mL	10–60
Eriodictyol			
Isoflavones *(20,163–165)*	Soy flour	75 g	60–135
Daidzein	Soybeans	200 g	40–180
Genestein	Boiled	100 g	8–70
Glycitein	Tofu		
Monomeric flavanols *(13,17,159)*	Chocolate	50 g	23–30
Catechin	Beans	200 g	70–100
Epicatechin	Apricot	200 g	20–50
	Cherry	200 g	10–44
	Green tea	200 mL	20–160

Note: The source, serving size and polyphenol content refers to the total content of the class of polyphenols (underlined) and not each individual ones.

2.2.2. Flavonols

Flavonols are the most ubiquitous flavonoids in foods with quercetin and kaempferol being the main representative ones. The richest sources are onions (up to 1.2 g/kg fresh weight), curly kale, leeks, broccoli, and blueberries (Table 1). These compounds are of interest because they inhibit histamine release *(16)*.

2.2.3. Flavanols

Flavanols are also called catechins. This group includes catechin, epicatechin, epicatechin gallate, and epigallocatechin gallate. Catechin and epicatechin are the main flavanols in fruits such as apricots and berries, in green tea and chocolate. Gallocatechin, epigallocatechin, and epigallocatechin gallate are found in certain types of leguminous plants, grapes and tea *(17,18)*.

2.2.4. Isoflavones

Isoflavones are flavonoids with structural similarities to estrogens. They are thought to have pseudohormonal properties because of their structural similarity to estradiol molecule and ability to bind to estrogen receptors; thus they are also classified as phytoestrogens *(8)*. Isoflavones are found abundantly in leguminous plants. Soya and its processed products are the main source of isoflavones in human diet. Soybeans contain between 580 and 3800 mg isoflavones/kg fresh weight and soymilk contains between 30 and 175 mg/L *(19,20)*.

2.3. Stilbenes

Stilbenes are important polyphenols present in grapes and berries. The most important stilbene is resveratrol. It is found in low quantities in wine (0.3–7 mg aglycones/L and 15 mg glycosides/L in red wine) *(21)*. Resveratrol is an important polyphenolic antioxidant with anti-inflammatory and anticarcarcinogenic properties *(22,23)*.

2.4. Lignans

Lignans are formed of two phenylpropane units. Linseed is the richest dietary source of lignans *(8)*. Leguminous plants (lentils), cereals (wheat), vegetables like asparagus, carrots and garlic, and fruits like pears and prunes are minor sources of lignans *(8)*.

3. VARIABILITY OF POLYPHENOL CONTENT OF FOODS

The concentration of various polyphenols in fruits and vegetables depends on many factors. Growth conditions such as the soil conditions and temperature, amount of light, fruit or vegetable species, type of cultivar, maturation stage at the time of harvest, storage time and temperature; all are important in determining the final composition of polyphenols in fruits and vegetables. In general, phenolic acid concentration decreases with ripening whereas anthocyanin concentrations increase. The type of cultivation could also influence the polyphenol content of the food. It has been shown recently in strawberries, blackberries, and corn that total polyphenol content is higher in the ones grown by organic or sustainable agriculture than those grown in conventional or hydroponic conditions *(24)*.

Methods of culinary preparation can also have a marked effect on the polyphenol content of the foods. For example, peeling fruits and vegetables can eliminate a significant

portion of polyphenols, as these phytochemicals are often present in higher concentrations in the outer parts than in the inner parts *(8)*. Onions and tomatoes lose 75 to 80% of their initial quercetin content after boiling for 15 min, 65% after cooking in microwave oven and 30% after frying *(25)*. Industrial food processing also affects polyphenol content of the foods. Because of the wide range of existing polyphenols and the considerable number of factors that can modify their concentration in foods; reference food composition tables for polyphenols do not exist for most fruits and vegetables.

4. CARDIOPROTECTIVE EFFECTS OF POLYPHENOLS

Various epidemiological studies have shown an inverse association between the consumption of polyphenols or polyphenol rich foods and the risk of cardiovascular diseases. This health benefit is clear in the "French Paradox" phenomenon as well as in the Mediterranean diets. The French paradox is defined as low incidence of coronary heart disease (CHD) even when consuming a diet high in saturated fats *(26)*. The Mediterranean diet, which is rich in fruits and wine, was shown to protect against the occurrence of CHD *(26,27)*. Hertog et al. *(28)* examined data from Zutphen Elderly Study and reported that high intake of dietary flavonols (particularly quercetin) was associated with a decreased risk of CHD. In a 10-yr follow up of the same group of elderly men, Hertog et al. *(29)* reported a decreased risk of mortality from all causes with increasing flavonol intake. A similar finding of decreased mortality with coronary disease with increasing flavonoid intake was reported from a Finnish cohort study *(30)*. A meta-analysis including 7 case-control and 10 cohort studies suggested a reduction of myocardial infraction risk by 11% with consumption of three cups of tea/d *(31,32)*. Results from these observational studies indicate improved cardiac health with increased intake of dietary polyphenols. Mechanisms by which polyphenols may be cardio-protective are discussed below.

4.1. Polyphenols as Antioxidants

Reactive oxygen species (ROS) from both endogenous and exogenous sources may be involved in the development of diverse human diseases, such as coronary artery disease, diabetes, and cancer *(33,34)*. Intracellular concentrations of ROS are generally modulated by the antioxidant defense systems of the cells. However, generation of large amounts of ROS can overwhelm the intracellular antioxidant defense system and cause activation of neutrophils, lipid peroxidation, protein modification, and DNA strand breaks. In vitro it has been shown that polyphenols, like catechin or quercetin can directly scavenge ROS such as, superoxide, hydrogen peroxide *(35)*, or hypochlorus acid *(36)*. The phenolic core of polyphenols can act as a buffer and capture electrons from ROS to render them less active *(37)*. Furthermore, polyphenols like quercetin can chelate metals like iron that can be involved in free radical formation *(38,39)*. Polyphenols can also interfere with cellular detoxification systems such as superoxide dismutase, catalase or glutathione peroxidases *(40,41)*. Polyphenols can also inhibit enzymes generating ROS like xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase *(39,42)*. In vitro investigation of tea flavonoids has shown that they are potentially strong antioxidants, up to five times more effective than vitamin C or E *(43)*.

Increased antioxidative capacity of human plasma is consistently observed in many studies over the hours following the intake of polyphenol rich beverages, such as tea

(44), wine *(45,46)*, and beer *(47)* as well as fruit and vegetables rich in polyphenols such as strawberries and spinach *(48)*. Antioxidant capacity of fruits and vegetables, cosmetics and supplements is expressed in Oxygen Radical Absorbance Capacity (ORAC) units. For example, blueberries have a high ORAC value (2400/100 g berries) where as black berries (2036/100 g), strawberries (1540/100 g), and raspberries (1220/100 g) have relatively lower ORAC values *(49)*. Only a couple of published reports have examined the effects of consuming fruits rich in polyphenols on the plasma ORAC. In a group of elderly women, consumption of a single bolus of either 240 g of strawberries or 294 g of spinach resulted in an increased total antioxidant capacity of urine by 9 or 27.1%, respectively, as determined by ORAC method *(48)*. Consumption of a single bolus of Bing sweet cherries by healthy women significantly reduced the plasma uric acid and increased vitamin C and total lipophilic ORAC within 5 h of the cherry consumption *(50)*. Results from these in vivo studies are consistent with those found in vitro.

Increased plasma antioxidant capacity after consumption of polyphenols could improve the ability of the body to protect cellular components like lipids and DNA from oxidative damage. Reduced concentrations of markers of DNA oxidation and lipid peroxidation were observed in humans after high intake (12 servings/d) of polyphenol rich fruits and vegetables *(51)*. Wines are naturally high in grape polyphenols, and a consumption of 240 mL of red wine/d for 2 mo showed pronounced reduction (approx 50% of basal values) in levels of oxidized DNA bases in blood leukocytes of healthy male volunteers, especially after consumption of a high-fat diet *(52)*. Also, in a group of 10 diabetic patients, consumption of 400 g of onions (rich in quercetin) and 6 cups of tea/d for 2 wk reduced the oxidizability of lymphocytic DNA by approx 16% *(53)*. On the other hand, no significant decrease in DNA oxidation in leukocytes was observed in 36 healthy subjects after consumption of 150 g of onions and 1 cup of black tea for 2 wk *(54)*. These discrepancies may be due to the type, amount and duration of the polyphenols fed in these studies.

4.2. Polyphenols and Blood Lipids

Reactive oxygen species produced intracellularly attack plasma low-density lipoproteins (LDL) and modify it to oxidized LDL (oxLDL). Oxidative modification of LDL is a key step in the development and pathogenesis of atherosclerosis *(55–57)*. oxLDL do so by attracting monocytes to the endothelium which eventually become foam cells and are incorporated into early atherosclerotic lesions *(58)*. Several in vitro studies strongly suggest that phenolic compounds protect LDL from oxidation by their ability to scavenge ROS *(59)*.

This protection of LDL from oxidative damage has also been shown in many human studies. Ingestion of approximately 7.7 mL/kg/d (approx 640 mL in an 80 kg man) of purple grape juice for 2 wk by patients with coronary artery disease reduced ex vivo susceptibility of LDL to oxidation by increasing lag time by 34.5% *(60)*. Similar effects were observed after consuming 10 mL/kg/d of concord grape juice for 2 wk by healthy adults *(61)*. Supplementation with 7 ml/kg/d of flavonoid rich cranberry juice for 2 wk in healthy men significantly reduced the oxidizability of LDL *(62)*. Tea polyphenols also inhibited LDL oxidation in some *(63)* but not other studies *(64)*. Consumption of either 1 g/d of nonalcoholic red wine extract (equivalent to 375 ml/d of red wine) or 30 mg/d of quercetin for 2 wk significantly reduced ex vivo susceptibility of LDL to

oxidation in a group of healthy male volunteers *(65)*. Significant reduction in LDL oxidizability was also observed after consuming 375 mL/d of red wine for 2 wk by healthy subjects *(66)*. In contrast, no protection against LDL oxidation was observed in a 4 wk cross-over study after consumption of black tea by a group of healthy volunteers *(67)*. A lack of protection against LDL oxidation was also observed after consumption of 550 mL of red or white wine for 4 wk by healthy subjects *(68)*. Similar results were obtained after supplementing 1.9 g/d of red wine phenolic compounds as pills for 2 wk in healthy subjects *(69)*. Different sources of polyphenols and different methods in assessing oxidizability of LDL, and the in vitro testing in the absence of serum may be some of the factors contributing to these inconsistencies *(70)*.

Elevated serum lipids are an important risk factor for development of atherosclerosis. Consumption of foods rich in polyphenols, such as red wine, elderberry juice, lyophilized grape powder, and chocolate increased high-density lipoprotein (HDL)- and decreased LDL-cholesterol and triglycerides in human volunteers who were obese or hyperlipidemic *(71–73)*. Reduction in plasma LDL-cholesterol and triglycerides in these studies was postulated due to reduced secretion of apo-B and VLDL packaging. In contrast to the results from these studies we did not find any change in serum lipids in study with healthy subjects who consumed 280 g/d of Bing sweet cherries for 28 d *(74)*. Also, a study involving wine consumption reported an increase in the post-prandial lipids in hypertryglyceridemic subjects, 6 h after consuming red wine *(75)*. Thus, it appears that the response of blood lipids to the consumption of polyphenols is also affected by the pre-existing blood lipids and the obesity related metabolic changes. In vitro, dealcoholized red wine has been shown to significantly up regulate LDL receptor activity in Hep G-2 cells *(76)*. This, increased uptake of plasma LDL by the liver could be a possible mechanism by which polyphenols reduce blood LDL.

4.3. Polyphenols and Endothelial Function

Endothelium not only functions as a permeability barrier but also controls vascular homeostasis; endothelial dysfunction can be associated with increased cardiovascular disease risk *(77)*. Dysfunction of vascular endothelium in humans can be measured by flow mediated dilation (FMD) of the brachial artery.

Acute consumption of 500 mL of red wine (0.8 g/kg ethanol) or dealcoholized red wine but not equal amounts of vodka (0.8 g/kg ethanol) improved FMD in healthy volunteers *(78)*. Consuming 250 mL of red wine or dealcoholized red wine while smoking one cigarette significantly attenuated reduction in FMD caused by cigarette smoking *(79)*. Thus, the positive effects of red wine on FMD should be attributed to polyphenols in wine rather than to alcohol. Consumption of a single dose of red or white wine (4 mL/kg) improved acute FMD, 60 min post-treatment in patients with coronary artery disease *(80)*. Improved FMD was reported in a similar group of patients after consuming between 4 and 8 mL/kg/d of purple grape juice for 4 wk *(81)*. Both short term (450 mL for 2 h) and long term (900 mL/d for 4wk) ingestion of black tea significantly improved FMD in 66 patients with coronary artery disease *(82)*. In a preliminary study with 10 young healthy volunteers, acute consumption of 450 mL of black tea significantly improved coronary circulation as determined by coronary flow velocity reserve, measured by the non invasive transthoracic Doppler echocardiography method *(83)*.

Supplementation of the diet with soy and other phytoestrogens has shown similar benefits on FMD. In a placebo controlled double blind study, supplementation with 54 mg/d of the phytoestrogen, genistein, for 6 months in postmenopausal women showed significant improvement in FMD *(84)*. A significant improvement in FMD was also observed in postmenopausal women with hypercholesterolemia after consuming 40 mg/d of isolated soy protein powder containing 80 mg of isoflavones for 4 wk *(85)*. Steinberg et al. *(86)* compared the effect of consuming 25 g of isolated soy protein with high (171 mg) or low (2 mg) isoflavone content in healthy postmenopausal women. After 6 wk, a nonsignificant trend for increased vasodilatory response was observed in the high-isoflavone group compared with the low-isoflavone or control group.

Consumption of 46 g of high flavonoid (213 mg procyanidin, 46 mg epicatechin) dark chocolate for 2 wk improved FMD in healthy subjects *(87)*. Thus, evidence from most human studies suggests improvement in endothelial function by various dietary polyphenols.

4.4. Polyphenols and Inflammation

Cardiovascular disease is an important inflammatory disease. Production of proinflammatory eicosanoids and cytokines plays an important role in the inflammatory process. Both cycloxygenase (COX) and lipoxygenase (LOX) are key enzymes in the conversion of arachidonic acid to the various eicosanoids involved in the process of inflammation. In vitro, polyphenols like quercetin have been shown to inhibit both COX and LOX enzymes *(88–90)*. COX-1 and COX-2 inhibition is the means by which aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) reduce inflammation. Anthocyanins from raspberries and sweet cherries inhibited COX-1 and COX-2 activities by 45 and 47%, respectively, when assayed at 125 μg/mL. Interestingly, cycloxygenase inhibitory activities of these anthocyanins was comparable to 10 μ*M* of the NSAID, ibuprofen and naproxen *(91)*. The grape polyphenol, reseveratrol, is thought to be anti-inflammatory because it inhibits prostaglandin biosynthesis *(92)*. Recent in vitro studies in polyphenols from cocoa have shown that epicatechin and proanthocyanidin have potent anti-inflammatory properties *(93)* and are potent inhibitors of 5-lipoxygenase, the enzyme responsible for production of pro-inflammatory leukotrienes *(94)*.

Inflammatory responses in the endothelial cells are an important step for initiating atherogenesis. An inflammatory response in endothelial cells attracts monocytes to the area and promotes their adhesion to endothelium. The adhered monocytes cross the intima, differentiate into macrophages and finally transform into foam cells, which are the characteristic components of early atherosclerotic lesions *(95)*.

Polyphenols are thought to prevent this cascade of events by preventing adhesion of monocytes to the endothelium. Consumption of 30 g ethanol/d as red wine (320 mL red wine/d) and not as gin (100 mL gin/d) by healthy men for 28 d, reduced by 96% tumor necrosis factor (TNF)-α induced ex vivo adhesion of monocytes to activated endothelial cells *(96)*. This effect may be because of down regulation of intercellular adhesion molecules (ICAM) on the monocyte surface *(96)*. In another study with 40 healthy men, serum concentration of adhesion molecules vascular cell adhesion molecule (VCAM)-1 and ICAM-1, and C-reactive protein (CRP) were reduced by 17 and 9 and a 21%, respectively, after consumption of 30 g ethanol/d as red wine for 28 d and not as gin *(97)*. Plasma levels of soluble adhesion molecules ICAM-1, VCAM-1, and E-selectin

were found to be elevated in a group of systemic sclerosis patients; their treatment with 100 mg/d of activin, a grape seed derived proanthocyanidin, for 1 mo significantly attenuated this increase in adhesion molecules *(98)*. Treatment of pre- and postmenopausal women with 36 g/d of lyophilized grape powder for 4 wk, significantly reduced plasma concentrations of the inflammatory cytokine TNF-α in both groups, but no change in interleukin (IL)-6 concentration was observed *(71)*. The transcription factor, nuclear factor (NF)-κB, is thought to be responsible for activating the genes responsible for the production of some cytokines, adhesion molecules and procoagulant proteins *(99)*. Acute consumption of moderate amount of red wine and not vodka with a high fat breakfast was shown to significantly decrease activation of NF-κB in peripheral blood mononuclear cells of healthy volunteers *(100)*. Together, these studies indicate that polyphenols reduce the production of inflammatory eicosanoids, adhesion molecules, and some cytokines.

We have found that consumption of (280 g/d) Bing sweet cherries by healthy men and women for 4 wk significantly reduced plasma concentrations of inflammatory markers like CRP, nitric oxide, and RANTES (Regulated upon Activation, Normal T-cell expressed, and secreted) *(74)*. CRP, which is an important marker of inflammation and also a risk factor for cardiovascular disease, decreased by 8 and 25% within 14 and 28 d respectively of consuming cherries (*see* Fig. 1A). After the discontinuation of cherry consumption for 28 d, plasma CRP levels increased but did not reach prestudy levels. Similarly, circulating NO which is primarily produced by iNOS and is a risk factor for atherosclerosis was reduced by 18% both after 14 and 28 d of consuming cherries (*see* Fig.1B). Plasma RANTES concentration decreased by 21% after 28 d of cherry supplementation and it continued to decrease even after the discontinuation of cherry consumption for 28 d (*see* Fig. 1C). Reduction in the plasma concentration of these inflammatory markers after consuming cherries may protect not only from CVD but from other inflammatory diseases like arthritis as well.

4.5. Polyphenols and Platelet Aggregation

Aggregation of platelets is known to contribute to the development of atherosclerosis and inhibition of platelet aggregation is regarded as beneficial *(101)*. Platelets produce the pro-inflammatory mediators such as thromboxane A2 (TXA2) and serotonin, which are key participants in atherogenesis *(102)*. In vitro, both quercetin (50–100 μmol/L) and catechin (50–100 μmol/L) inhibited collagen induced platelet aggregation and platelet adhesion to collagen; both polyphenols used in combination showed similar results but at much lower concentration (quercetin 5 μmol/L, and catechin 25 μmol/L) *(103)*. Increased efficacy of these polyphenols when added together, suggests a synergistic effect.

Prevention of platelet aggregation by polyphenols has also been shown in human intervention studies. Supplementation with 234 mg/d of cocoa flavanols and procyanidins in healthy subjects for 28 d significantly increased plasma epicatechin and catechin levels and decreased collagen induced platelet aggregation *(104)*. Consumption of 37 g of high-procyanidin (4 mg/g) chocolate by healthy volunteers increased plasma prostacyclin by 37% and decreased plasma leukotrienes by 29% after 2 h of consumption *(105)*. This decrease in plasma prostacyclin:leukotrienes ratio can decrease platelet activation in humans. Several studies done in vitro and in animal models reported

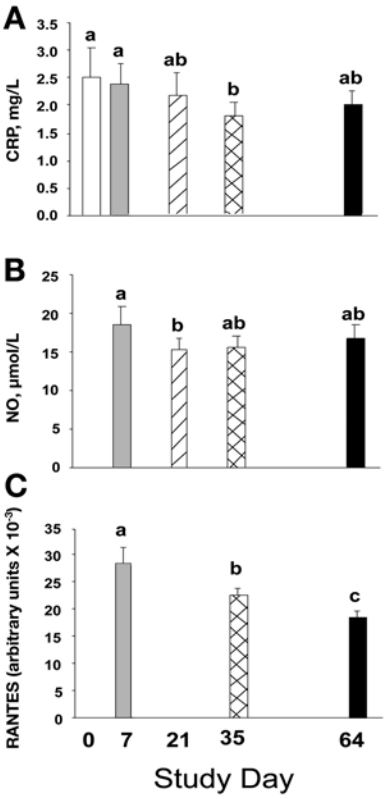

Fig. 1. (**A**) Effect of consuming Bing sweet cherries on circulating concentrations of CRP, (**B**) NO, and (**C**) RANTES in healthy humans. Cherries (280 g/d) were consumed between study days 7 and 35 and not during the rest of the study days. In **A** and **B** values are means and SEM ($n = 18$). In **C**, $n = 3$ pooled plasma samples from 18 subjects (6 subjects/pool). Bars with different superscripts are significantly different ($p < 0.05$). Reproduced with permission from ref. *74*.

beneficial effects of red wine and other polyphenol rich fractions from grapes on platelet aggregation *(106,107)*. In humans a reduced ex vivo platelet aggregation after consuming 5 to 7.5 mL/kg/d of purple grape juice for 7 to 10 d was shown, but no effect was observed with consumption of equivalent amounts of orange or grapefruit juice *(108)*. Thus, some of the dietary polyphenols may aid in reducing platelet aggregation and prevent clot formation.

5. POLYPHENOLS AND CANCER

Cancer is one of the biggest health problems facing American society with a steep age dependent increase. The incidence of some cancers has increased over the past several years whereas others have decreased. Willet suggested that around 32% of cancers could be prevented by dietary modification *(109)*. Most of the epidemiological studies have indicated inverse associations between several forms of cancer and the intake of polyphenol rich fruits and vegetables.

Flavonoid intake was evaluated in the Zutphen Elderly Study with respect to cancer incidence *(110,111)*. No association between flavanol intake and total cancer mortality

was observed in these elderly men *(110)*. In a 10-yr follow up of the same cohort, a slight inverse association between non tea catechins intake and lung cancer incidence was reported *(111)*. In a cohort study of 9959 men and women, an inverse association between the intake of flavonoids and incidence of all cancers combined was reported; association was most apparent for lung cancer *(112)*. In contrast, in a cohort study on diet and cancer, flavonol intake was not associated with incidence of stomach cancer, lung cancer or colon cancer after 4 yr of follow up *(113)*. This follow-up period may be too short to see any significant effects, a longer period may be necessary.

Case control studies in humans have also examined the relationship between dietary polyphenols and cancer. De Stefani et al. *(114)* in a study from Uruguay analyzed dietary data from 541 individuals with lung cancer and 540 hospitalized controls and reported a significant inverse association of lung cancer risk with increasing carotenoid, glutathione, flavonoid, and vitamin E intake. In a case control study in Spain which included 354 gastric cancer cases and 354 matched controls, an inverse association was observed with total flavonoid intake and gastric cancer risk; kaempferol and quercetin in particular were reported to be protective *(115)*. In a large cohort of 34,651 postmenopausal women, total catechin intake was inversely associated with risk of rectal cancer *(116)*. Intake of catechins from fruits but not tea was inversely associated with a lower risk of cancer of the upper digestive tract in the same cohort. Thus, it is not clear if the anti-cancer effects of fruits are due to their phenolic or non-phenolic components like fiber and other nutrients.

It has been suggested that relatively higher consumption of soy foods may contribute to the relatively low rates of breast, colon, and prostate cancers in countries such as China and Japan *(117)*. Soy and other legumes are important source of isoflavonoid phytoestrogens, genistien and diadzein. In a case control study in postmenopausal women with diagnosed breast cancer, a trend towards lower urinary diadzein and genistein was reported compared with controls *(118)*. A significantly lower urinary excretion of lignans was observed in patients with breast cancer compared with normal omnivores and vegetarians *(119)*. However, in a group of postmenopausal women, 88 with breast cancer and 298 controls, higher urinary excretion of genistein was weakly but non-significantly associated with reduced risk of cancer *(120)*. In a clinical trial with 10 women, consumption of a soy-containing diet providing an average of 154 mg/d of isoflavones for 1 mo resulted in a significant decrease in plasma levels of estradiol and progesterone, two biomarkers of breast cancer risk *(121)*. Consumption of isoflavone rich soy products seems to be specifically protective against hormone related cancers like breast cancer. In a case control study of 83 Caucasian prostrate cancer cases and 107 controls, increased consumption of genistein was shown to be slightly protective against prostrate cancer, but the association was not significant *(122)*. No association between prostrate cancer risk and serum concentrations of enterolactone, a type of lignan, was found in another case control study *(123)*.

Thus, epidemiological data do not demonstrate a clear negative association between the intake of polyphenols and incidence of cancer as observed with the consumption of fruits and vegetables. This lack of association may be real or may be because of lack of adequate measures to assess polyphenol intake by most food frequency questionnaires used in the epidemiological studies which are not specifically designed to assess phytochemical intake. More interventional clinical trials are needed to confirm the

anticarcinogenic effects of various polyphenols. However, the protective effects of increased fruit and vegetable intake are well documented.

6. POLYPHENOLS AND NEURODEGENERATIVE DISEASES

Neurodegenerative diseases represent an increasing burden on our ageing societies. About 15% of the population over 65 is afflicted by Alzheimer's disease and 1% by Parkinson's disease, not including other types of dementia resulting from ischemic injury *(124)*. Oxidative damage of neuronal biomolecules, accumulation of iron in specific brain areas and inflammatory processes are major pathological processes of these neurodegenerative disorders *(125)*. Several studies in rats and mice have shown the possibility of reversing age related effects when these animals were fed diets rich in berry extracts, particularly blueberries *(126,127)*. A diet rich in blueberry extract improved short term memory and reversed some loss of motor behavioral defects in ageing rats *(128,129)*. Similar results were obtained from aqueous extracts of spinach *(128)*. Cognitive performance in old rats, including Morris-water maze, which measures special learning and memory, correlated with anthocyanin levels found in the cortex of these rats when fed a diet containing blueberry extract *(130)*. Intravenous injection of epicatechin or catechin to mice improved the memory impairment induced by cerebral ischemia *(131)*. Dietary supplementation with grape polyphenols reduced neurodegenerative changes induced by chronic ethanol consumption, and improved the synaptic function measured on isolated synaptosomes *(132)*. In vitro, catechins have been shown to improve the survival of cultured neuronal cells when challenged by a β-amyloid peptide, a compound postulated to be involved in the pathogenesis of Alzheimer's disease *(133)*, or oxidized LDL *(134,135)*. These effects seem to be mediated by restoration of protein kinase C or inhibition of NF-κB translocation, both of which are involved in the regulation of cell proliferation and apoptosis.

The relationship between polyphenols and neurodegenerative diseases is a relatively very new area of investigation and thus, very few epidemiological studies have been completed. In a prospective study in elderly population (age 65 and over), moderate consumption of wine (3–4 standard glasses/d) was negatively associated with the risk of incident dementia *(136)*. In a case control study, monthly and weekly intake of wine was significantly associated with lower risk of dementia whereas monthly intake of beer was significantly associated with increased risk in a cohort aged 65 and above *(137)*. In a prospective analysis, wine consumption along with other factors like coffee consumption, use of NSAID and physical activity were associated with reduced risk of Alzheimer's in people 65 yr and above *(138)*. In a large cohort study with subjects aged 65 and above, intake of antioxidant rich flavonoids was inversely associated with risk of incident dementia *(139)*. Most of the epidemiological studies suggest the potential benefit of polyphenols against neurodegenerative diseases; however further intervention studies are needed to assess the benefits and any potential risks.

7. POLYPHENOLS AND INFECTIONS

Berries, in particular cranberries, have been shown to have antibacterial properties which make them of potential interest for several applications *(140)*. The major antibacterial mechanism appears to be interference with the attachment of bacteria to the cell

surfaces *(141)*. The attachment of bacteria can be inhibited when both fructose and a high-molecular-weight proanthocyanidin are present *(142)*. Such a combination of fructose (present in all fruits) and the particular proanthocyanidin is present in cranberries. Inhibition of adherence is important in preventing several bacterial infections, and because these compounds are not bactericidal, selection of resistant strains is unlikely *(143)*. This is an important consideration as resistance of pathogenic bacteria to antibiotics is increasing. Several in vitro studies have shown the potential of cranberry juice to prevent urinary tract infections *(144)*. The original anecdotal use of cranberry juice was based on the presumption that it reduces the pH of urine, but more recent data indicate that the effect is mediated by reduced cell adhesion *(141)*. Avorn et al. *(145)* showed significant decrease in the incidence of bacteriuria and pyuria in a group of elderly population after consuming 300 mL/d of cranberry juice for 6 mo. Reid et al. *(146)* reported significant reduction in bacterial biofilm load in the urine of patients with spinal chord injuries after consuming 750 mL of cranberry juice daily for 1 wk compared with when these patients had equivalent amounts of water. Adhesion of bacteria to tooth enamel to form a biofilm is the first step towards development of dental plaque which eventually causes cavities and gum disease if untreated. Usage of mouth wash containing nondialyzable material (NDM) from cranberries for 6 wk, twice daily, significantly reduced salivary *Streptococcus* mutant counts as well as the total bacterial counts in healthy volunteers; in vitro the effect was shown to be mediated by reduced adhesion of bacteria to the teeth by the NDM from cranberries *(147)*. Incubation of *Helicobacter pylori* for 18 h with extracts of raspberry, strawberry, elderberry, cranberry, and blueberry significantly inhibited growth of the bacteria *(148)*. More information about the role of berries in infections and other chronic diseases has been published in a recent comprehensive report *(9)*. Although more work needs to be done to accurately identify the polyphenols that have the ability to prevent infections, consumption of polyphenol rich fruits and vegetables is a desirable way to prevent infections without the complications of antibiotic resistance.

8. PURE COMPOUNDS OR WHOLE FRUIT

The consumption of fruits and vegetables has consistently demonstrated protective effects against many health problems as discussed above. Yet, pure compounds isolated from these fruits and vegetables have not demonstrated same preventive efficacies *(149,150)*.

This disconnect between epidemiology and clinical studies may be the result of consumption of high amounts of single compounds in isolation in these studies which has rarely the same effects as the biologically complex mixture of compounds present naturally in fruits and vegetables. For example, Vitamin C has been shown to be a potent antioxidant, but when given at doses higher than 500 mg/d, it has been shown to be a pro-oxidant and caused increased DNA damage. Liu *(151)* concluded that the additive and synergistic effects of the phytochemicals present in fruits and vegetables are responsible for their potent health benefits. Thus dietary supplements cannot replace fruits and vegetables for achieving the health benefits seen from fruit and vegetable consumption.

9. CONCLUSIONS

Studies reviewed here indicate a reduction in chronic inflammatory diseases with the increased consumption of foods rich in polyphenols. Therefore, the production and

consumption of such foods needs to be increased. Further studies are needed to determine the health benefits of polyphenols in human populations with increased inflammation such as CVD or arthritis, effectiveness of purified polyphenols vs mixtures from food sources, and processed vs fresh fruits. Studies are also needed to understand the mechanisms underlying the health benefits of such foods.

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28 Polyphenols and Immunity

Tirang R. Neyestani

Abstract

Polyphenols are the most abundant antioxidants in our diet. The pro-oxidant/antioxidant balance is believed to be an important determinant of the immune cell function as the rather high percentage of polyunsaturated fatty acids (PUFAs) in their cell membranes has made the immune cells particularly sensitive to oxidative stress. Polyphenols as dietary antioxidants may affect various aspects of both innate and adaptive wings of the immune system by shifting pro-oxidant/antioxidant balance toward antioxidant. Complement system, for instance, has been shown to be inhibited by polyphenols and this complement inhibitory effect may have some role in anti-inflammatory properties of polyphenols. The anti-inflammatory effects of polyphenols, which may be exerted at the molecular level, are likely to be dependent on the specific structure of polyphenolic compounds. Macrophage functions, including cytokine production, may also be affected by some flavonoids through modulation of inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). Many experimental studies have reported immune modulating effects of polyphenolic compounds on both humoral and cell-mediated adaptive immunity. On the other hand, oxidative stress has been proposed as to play a role in many clinical conditions including allergy, cancer, and atherosclerosis, among others. Many experimental as well as human studies have supported the prophylactic effects of polyphenols against these pathologies. These data altogether suggest that the effects are exerted through antioxidant-mediated immune modulation mechanisms. On the other hand, polyphenolic compounds may act as a pro-oxidant under certain situations and this may in part explain the inconsistencies often seen in results of different studies. Finally, the results of most, notably in vitro, studies on polyphenols and immunity can be hardly interpreted as to long-term general health of human. Therefore, further laboratory and clinical studies are still needed to clarify the effects of polyphenolic compounds on the immune function as well as on health status.

Key Words: Polyphenols; antioxidant; immunity.

1. INTRODUCTION

Polyphenols are plant molecules entering our bodies through diet. The relationship between polyphenol-rich food consumption and reduced possibility of being affected by some diseases has attracted increasing interest from consumers, food manufacturers and nutritional scientists. Fruit and vegetable consumption may prevent cancers *(1)* and stroke *(2)*, whereas wine consumption may have similar effect in preventing coronary heart disease (CHD) *(3,4)*, and prostate cancer *(5)*. Soy consumption may have protective effects against cancerous cells and osteoporosis *(6,7)* and tea polyphenols may prevent different cancers *(6,8)* and arthritis *(9)*.

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The possible therapeutic effects of polyphenols against some parasitic *(10)* bacterial *(11)*, viral, and fungal *(12)* agents have been proposed. The anti-histaminic effect of polyphenols is the other interesting field having evaluated by some investigators *(13–16)*.

Considering the fact that the immune system is critical to the establishment and maintenance of good health by providing a first line of defense against infection and neoplasia and by contributing to overall homeostasis *(17,18)*, the subject comes to mind that polyphenols may have some immune enhancing effects on one hand, and antipathogenic effects directly on pathogens, on the other. Looking at the roles of oxidative stress in a variety of human diseases *(19)*, the effects of antioxidants on immune function *(20)*, and antioxidant properties of polyphenols *(21)* it is likely that polyphenols exert their immune enhancing effects mostly by acting against oxidative stress.

2. POLYPHENOLS: DEFINITION AND CLASSIFICATION

A phenolic molecule is of plant origin. It is therefore impossible to know precisely the nature of all of the polyphenols that we ingest. Polyphenols are reducing agents, and together with other dietary reducing agents, such as vitamin C, vitamin E, and carotenoids, they protect the body's tissues against oxidative stress. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular disease (CVD), inflammation, and others. Indeed, polyphenols are the most abundant antioxidants in our diet *(21)*. The chemical structure of a polyphenol will affect its biological properties: bioavailability, antioxidant activity, specific interactions with cell receptors and enzymes among the others.

The main classes of polyphenols are defined according to the nature of their carbon skeleton: phenolic acids, flavonoids and the less common stilbenes, and lignans. Phenolic acids are abundant in foods. The most frequent encountered are caffeic acid and, to a lesser extent, ferulic acid. Flavonoids, the most abundant polyphenols in our diets, can be divided into several classes according to the degree of oxidation of the oxygen heterocyclic: flavones, flavonols, isoflavone, anthocyanins, flavonols, proanthocyanidins, and flavanones. The main source of isoflavones is soy, which contains about 1 mg of genistein and daidzein/g dry bean *(21,22)*. These two isoflavones have estrogenic properties so they may have a role in prevention of breast cancer and osteoporosis *(7)*.

The structural diversity of dietary polyphenols is not limited to differences in the structure of the carbon skeleton and in the oxidation state of the heterocyclic of flavonoids. It is further complicated by varying patterns of hydroxylation of the phenolic rings, by glycosylation of most flavonoids, by acylation with phenolic acids and by the existence of stereoisomers, among others *(21)*.

3. THE IMMUNE SYSTEM

To endure the hazards of existence, the individual needs to be defended. Evolution of the species has generated various physiological systems that defend but two systems bolster innate defense with individual experience: the nervous and the immune. The nervous system keeps us out of trouble by its organs that sense, see, smell, and hear, and by its brain that learns, anticipates, and plans. The immune system defends us against dangers that are beyond the knowledge of the nervous system: infectious agents, foreign

cells and molecules, and abnormal cell arising within our own bodies. Like the nervous system, the individual immune system learns and remembers *(23)*.

The immune system is a complex system of molecules, cells, and tissues widely dispersed throughout the body, which interact in a coordinated and orchestrated manner to control and eliminate infectious agents, malignant and transformed cells, and other unwanted antigens. At the cellular level, the immune system is composed of various types of cells. Macrophages and related cells engulf and degrade bacteria and other antigens and present degraded fragments of them to bone marrow-derived (B) and thymus-derived (T) cells, the two classes of lymphocytes are responsible for specific immune function. Natural killer (NK) cells nonspecifically recognize and destroy transformed and virus-infected cells *(18)*.

The immune system is quiescent until antigenically challenged. Once challenged, the natural immune response is initiated through the activation of macrophages and NK cells and the production of various associated soluble factors. With sufficient antigenic stimulation of lymphocytes, an acquired immune response is triggered, resulting in the clearance of the stimulating antigen and in the generation of memory cells (primary response) that will, upon subsequent challenge by the same antigen, lead to its more rapid and efficacious clearance (secondary response) *(17)*. The immune response can thus be divided into two types: (i) adaptive immunity, which develops through primary antigen encounter and sensitization and is enhanced through repetition of stimuli, and (ii) nonadaptive immunity, often called natural immunity, which does not require sensitization and is not enhanced by repetition. Physical barriers such as skin and mucous membranes that protect the internal environment of human body, are also considered as components of natural or innate immunity.

Both natural immunity and adaptive immunity consist of cellular and humoral components (*see* Fig. 1). It should be re-emphasized that all of these components work coordinately and intimately. Phagocytic cells and NK cells are among the cellular components of natural immunity while their cytokines such as interleukin (IL)-1, and tumor necrosis factor-α (TNF-α) play their roles in natural immunity as humoral factors *(24)*. Lymphocytes have undoubtedly the key role in adaptive immunity. Whereas B cells may act as an antigen-presenting cell (APC), both T cells and B cells are highly differentiated upon repeated antigenic stimulation.

Based on cellular markers, T cells can be divided into two main subsets: (i) helper/inducer cells, which are $CD4^+$, and (ii) suppressive/cytotoxic cells, which are $CD8^+$. It has been shown that T-helper (Th) cells can be further divided into Th-1 and Th-2 cells *(25)*. Some $CD4^+$ cells, which have another cellular marker, $CD25^+$, are referred to as regulatory T-(reg) cells. T-(reg) cells have important roles in the immunity against infections *(26)*. These cellular subsets have different cytokine profiles and hence various functional and physiological roles. Whereas Th-1 cells may secrete interferon γ (IFN-γ), Th-2 cells may release different cytokines such as IL-4, IL-5, and IL-13.

Th-1/Th-2 balance is very important as in some autoimmune disorders such as type 1 diabetes mellitus (DM), systemic lupus erythematous (SLE) and rheumatoid arthritis (RA) the balance has been demonstrated to be shifted toward Th-1, whereas in allergic reactions and parasitic infections, Th-2 arm predominates *(27)*. Therefore, the balance and occasionally the predominance of one of these two arms (i.e., Th-1 and Th-2) may

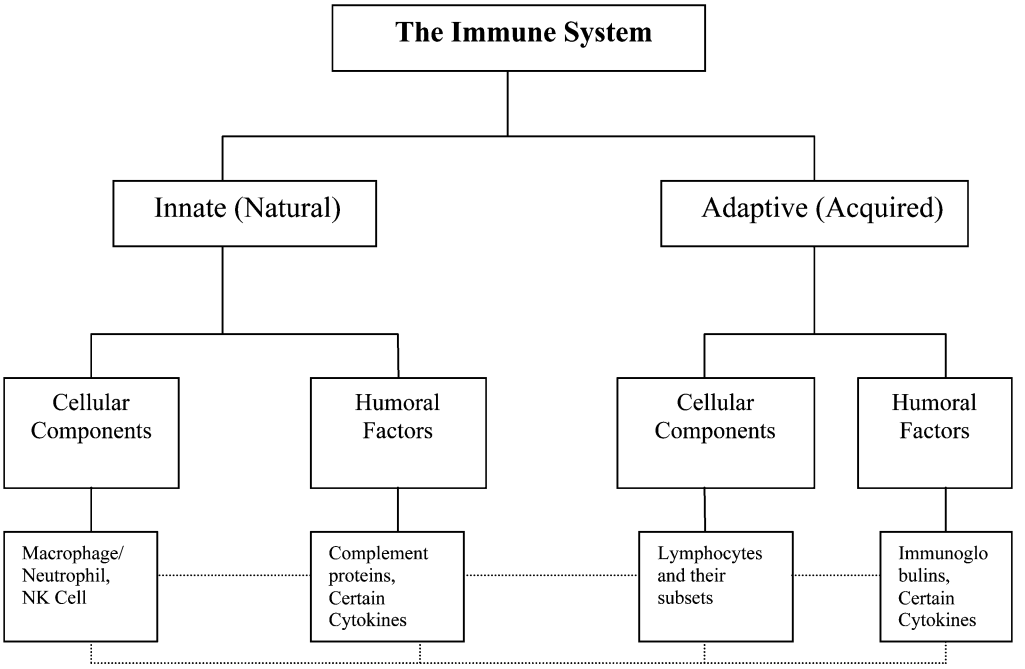

Fig. 1. Schematic representation of major cellular and humoral components of the immune system. All these components are interconnected (dotted). Physical barriers are also considered as parts of innate immunity (not shown).

have a crucial role in keeping health and determining the pathogenesis as well as the outcome of disease. Antigen-stimulated B-cells may also be differentiated into antibody-secreting plasma cells. Immunoglobulins may be divided to five isotypes including immunoglobulin (Ig) G, IgM, IgE, IgA, and IgD. A subgroup of both T- and B-cells will differentiate into memory cells, with highly specialized T-cell receptors (TCR) and surface immunoglobulins, respectively *(28,29)*.

4. OXIDATIVE STRESS AND IMMUNE FUNCTION

Chemical compounds contain two or more elements that are bound together by a chemical bond. In most instances, the bonding involves negatively charged electrons. The arrangement of the electrons determines the stability of the compound. A stable compound has electrons that are paired. If an electron is unpaired, the molecule becomes more reactive and unstable than it used to be. A compound or element with one or more unpaired electrons is referred to as a free radical. To stabilize itself, a free radical may attack other molecules and abstracts an electron from them and thus making them new free radicals. This chain reaction will continue until the free radical couples with another molecule containing unpaired electron or it is quenched by the action of other biologically active molecules called antioxidants *(20)*.

Oxygen is essential to aerobic life, but a part of the consumed oxygen turns into reactive oxygen species (ROS). For example, the superoxide anion (O_2^-) may be generated in many cell redox systems, such as those involving xanthine oxidase, aldehyde oxidase, membrane-associated NADPH oxidases and the cytochrome P-450 system. It is estimated that about 1 to 4% of the total oxygen taken up by mitochondria may be used for O_2^- production, and about 20% of this may be ejected into the cell. Stimulated macrophages and monocytes also release large amounts of O_2^-. This radical is not very reactive and that is why it can diffuse rather large distance through the cell, where it is converted in a metal catalyzed reaction into the more reactive hydroxyl radical ($OH^\bullet$). Potentially injurious free radicals present in pollutants, halogenated anesthetics and cigarette smoke may have a role in disease pathogenesis not only in adulthood but in infancy and childhood as well *(30,31)*.

To scavenge or neutralize ROS, aerobic organisms have evolved a variety of systems including low molecular weight antioxidants like α-tocopherol, ascorbic acid, gluthatione, and antioxidant enzymes such as superoxide dismutase (SOD), catalase and peroxidases. The imbalance in oxidant/antioxidant system shifted towards oxidant is referred to as oxidative stress. This is a potentially harmful phenomenon since ROS can interact with lipids, proteins and DNA, which may eventually result in mutation, neoplastic transformation, loss of cellular function, and, if progressed enough, cell death.

The antioxidant systems work coordinately as "defense systems" against oxidant-induced tissue injury, but may also modulate ROS-sensitive signal transduction pathways *(30)*. Oxidative stress may result in suppression of IL-2 production, protein tyrosine phosphorylation, and reduced intracellular calcium mobilization, low-DNA binding activity of nuclear transcription factors, NFAT, and nuclear factor-κB (NF-κB), and increased binding activity of activating protein-1 (AP-1). Treatment of oxidatively stressed cells with an antioxidant, *N*-acetylcysteine, can reverse these changes *(32)*.

Though free radicals may be potentially injurious to the cells, their production has a physiological philosophy behind. In fact, certain immune cells, mostly neutrophils and macrophages, use free radicals as weapons to destroy invading pathogens *(20)*. Free radicals are therefore like a two-edged sword that, if not subtly controlled by antioxidant defense mechanisms, may damage self-cells and tissues.

The pro-oxidant/antioxidant balance is an important determinant of the immune cell function, not only for maintaining of integrity and functionality of membrane lipids, cellular proteins, and nucleic acids of the immune cell, but for the control of signal transduction and gene expression as well. The immune cells are particularly sensitive to oxidative stress because of the rather high percentage of polyunsaturated fatty acids (PUFAs) in their cell membranes. On the other hand, these cells are frequently exposed to this stress because of the free radical production as a part of their normal function *(33)*. To overcome this problem, the immune cells usually contain higher amounts of antioxidants than do the other cells *(33,34)*.

Polyphenols as dietary antioxidants may affect various aspects of the immune system by shifting pro-oxidant/antioxidant balance towards antioxidant. This subject is reviewed briefly in this chapter.

5. INNATE IMMUNITY

5.1. Physical Barriers

Many tissues and cells, including skin and mucous membranes, obtain polyphenols through blood circulation. Actually the blood concentration of polyphenols is relatively low. For example, plasma concentrations of flavonoids, even in the populations consuming large amounts of plant material, are around 1 μ*M* but it has been shown that some cell types accumulate certain flavonoids *(35)*. It is still unknown whether skin and mucosal cells have such a characteristic. The effects of systemic polyphenols on skin and mucous membranes have not been addressed yet but some of these compounds may be topically effective in treatment of skin and mucous membrane inflammation, especially in the form of hydrophilic cream *(36)*.

5.2. Humoral Factors: Complement System

The complement system is comprised of a number of serum and membrane-bound proteins that play an important role in the elimination of foreign microorganisms while protecting the host organism from complement-related damage. Complement system may be activated through classical, alternative or lectin pathway. Once activated, an orchestrated series of biochemical reactions will lead to cell lysis, opsonization, or chemotaxis. The activation of complement is therefore crucial in occurrence of inflammation *(37)*.

The possible effects of dietary polyphenols on complement system have not been fully investigated. In a study on allergenic plant pollen extracts, which are widely used in clinical practice for diagnostic as well as therapeutic purposes, it was demonstrated that such extracts are capable of consuming complement in every human serum, independent of the clinical condition. The capacity of distinct pollen extracts to inactivate hemolytic complement was dependent on the plant species and the most potent extracts were of the weeds and trees. The ultraviolet (UV) spectroscopy analysis showed that flavonoids were firmly bound to the allergenic proteins. It was therefore speculated that complement inactivation by allergenic and nonallergenic pollen extracts was due to polyphenolic (flavonoid) structures bound to the pollen proteins *(38)*. In another experiment, complement-modulating properties as well as antimicrobial and superoxide scavenging effects of a series of dimeric procyanidins (1–9) were evaluated. Only the compounds with orthotrihydroxyl groups in the B-ring exhibited inhibitory effects on complement classical pathway *(39)*. The specific effect of individual polyphenolic compounds on complement was further demonstrated in a study on eight antioxidants from five different polyphenolic classes (cinnamic acids, benzoic acids, flavonoids, proanthocyanidins and stilbenes) for their antioxidant activities as well as complement modulating activities in vitro. Though procyanidin C1 was found to be a strong inhibitor of lipid peroxidation and the classical pathway of the complement system, genistein exhibited a very low antioxidant activity, a high cytotoxicity and a low complement-inhibiting activity *(40)*. The anti-complement activities of certain polyphenols have been shown in some other studies *(41–44)*. Complement inhibitory effects may have some role in anti-inflammatory properties of polyphenols. These effects are likely to be dependent on the specific structure of polyphenolic compounds.

5.3. Cellular Components: Phagocytes (Macrophages, Neutrophils), Natural Killer Cells

Various polyphenolic compounds may have different effects on macrophage functions, depending on their structure, dose and duration of consumption. Red grape juice and red wine are among the dietary sources of such polyphenols as resveratrol. However, consuming a single dose of 500 mL of red wine (12% ethanol), a 12% ethanol dilution, dealcoholized red wine, and red grape juice did not affect phagocytic activity and intensity of neutrophils and monocytes, production of TNF-α, IL-2, IL-4, and activity of NK cells *(45)*. When the effects of resveratrol and quercetin on intracellular killing of a pathogenic fungus, *Candida albicans*, in macrophage-like cells (U937 human promonocytic cell line) were compared, intracellular killing was decreased by both quercetin and resveratrol at 10 μ*M* concentration but was enhanced by 1 μ*M* resveratrol, after 20 h of treatment. Phagocytosis rate, expressed as phagocytosis frequency (i.e., percentage number of phagocytosing cells/total cells), at 20 h was highest with 10 μ*M* resveratrol and was higher with 10 μ*M* quercetin than with 1 μ*M* resveratrol *(46)*.

Interestingly, both polyphenols showed cytostatic activity. Flow cytometric analysis demonstrated resveratrol-induced apoptosis after 4 h incubation and at concentrations as low as 1 μ*M* and 100 n*M*. Another interesting finding was that resveratrol- or quercetin-treated, but unstimulated, cells did not express TNF-α protein. These findings suggest that wine polyphenols, at the same concentrations as those found in plasma after moderate wine consumption, have immunomodulating effects on cellular components of natural nonspecific immunity and that these effects may be found of clinical applications in anti-infective, anti-inflammatory, and anti-cancer therapies *(46)*. Recently, it has been proposed that the anti-inflammatory effects of resveratrol may be exerted through both NF-κB-dependent *(47)* and NF-κB-independent pathways *(48)*.

The inhibitory effects of polyphenols on phagocytosis are seemingly dependent on their anti-oxidative properties. In an in vitro experiment on the effects of flavonoids on myelin phagocytosis by macrophages, luteolin, quercetin, and fisetin significantly decreased the amount of myelin phagocytosed by a macrophage cell line without affecting its viability. The inhibitory concentration [IC (50)] values for these compounds ranged from 20 to 80 μ*M*. Here again this inhibitory effect was found to be dependent on structure and antioxidant activity of the flavonoid. Those flavonoids with hydroxyl groups at the B-3 positions in combination with a C-2, three double bonds and with higher antioxidant activity were most effective. The fact that reactive oxygen species are required for phagocytosis by macrophages may partly explain the correlation between the capacity of various flavonoids to inhibit phagocytosis and their antioxidant activities. These polyphenolic compounds may therefore be found useful in limiting the demyelination process in such demyelinating disorders as multiple sclerosis *(49)*.

Though some flavonoids may have inhibitory effects on macrophage secretory function, certain polyphenols may act in a reverse direction. This concept was studied in lipopolysaccharide (LPS)/IFN-γ activated RAW 264.7 macrophages. While quercetin and genistein inhibited TNF-α production, kaempferol, myricetin, and daidzein induced TNF-α formation. Anthocyanidins/anthocyanins and anthocyanin-rich extracts also showed inhibitory effects on TNF-α secretion and acted as modulators of the immune response in activated macrophages. Chlorogenic acid had no effect on TNF-α production.

Of interest is that the effect of some polyphenols may be changed by glycosylation, as glycosylated genistein acted in contrast to genistein and inhibited TNF-α production. Glycosylation of daidzein had no effect on its activity *(50)*.

The biological effects on macrophage of some polyphenols, which may have different polymeric forms, may be influenced by degree of polymerization. For example, procyanidins, which are found in cocoa liquor and are potent antioxidants and may act as anti-inflammatory agents, may have different degree of polymerization. Though cocoa and isolated procyanidin fractions (monomer through decamer) may modulate mRNA expression of IL-1β, the pro-inflammatory cytokine, in phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs), this effect is apparently influenced by the length of the molecule (i.e., the smaller fractions of cocoa [monomer–tetramer] reduce IL-1β expression in PHA-stimulated PBMCs by 1–15%, whereas the larger oligomers (pentamer–decamer) increase expression by 4-52% *(51)*.

From the mechanistic point of view, the anti-inflammatory effects of some polyphenols such as theaflavin-3, 3′- digallate from black tea are exerted by suppressing the activation of NF-κ-B through down-regulation of Iκ-B kinase (IKK) activity in macrophage *(52)*. Polyphenols may also have some effects on arachedonic acid cascade and prostaglandin (PG) biosynthesis. As resveratrol was found to impair cyclooxygenase-2 (COX-2) induction stimulated by LPS and phorbol myrestate acetate (PMA) or by $O2^{-}$ or H2O2 exposure in murine resident peritoneal macrophages. These effects of resveratrol on arachedonic acid release and COX-2 over-expression were correlated with a marked reduction of PG biosynthesis *(53)*.

Macrophage functions may be affected by apigenin and related flavonoids through modulation of inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). In LPS-activated RAW 264.7 macrophages, apigenin, genistein, and kaempferol were found to be active inhibitors of transcriptional activation of COX-2, with $IC_{50} < 15$ μ*M*. Apigenin and kaempferol showed inhibitory effects on transcriptional activation of iNOS, with $IC_{50} < 15$ μ*M*. Apigenin also blocked the LPS-induced activation of NF-κB *(54)*. Aarachidonate-derived PGs promote inflammatory reactions *(55)*. Cytokine production of macrophages may also be influenced by some polyphenols. Resveratrol, for instance, has been reported to enhance TNF-α, IL-12, and IL-1β production from LPS-activated phorbol myristate acetate (PMA)-differentiated THP-1 human macrophages. However, cytokine production may differ with IFN-γ-primed macrophages. As, in this situation, resveratrol suppressed the expression of HLA-ABC, HLA-DR, CD80, CD86 and inhibited production of TNF-α, IL-12, IL-6, and IL-1β induced by LPS. The differential effect of resveratrol on expression of CD14, LPS-binding protein, might be related to differential response of macrophages to LPS with or without IFN-γ priming *(56)*. Similar effects of resveratrol on expression of costimulatory molecules (e.g., CD80 and CD86) and HLA classes I and II in murine bone marrow-derived dendritic cells (BMDCs) has also been reported *(57)*. Down regulation of DC differentiation and maturation may be clinically useful in chronic inflammatory diseases. Some clinical trials, however, failed to show immunomodulating effects of acute resveratrol rich beverages intake in healthy men *(58)*.

Immune cell apoptosis is another possible mechanism of polyphenol immunomodulation. In an in vitro study, EGCG, and ECG were found to induce apoptosis in monocytes.

ECGC, in particular, activated caspases 3, 8, and 9, which play a central role in the apoptotic cascade, in a dose-dependent manner. Interestingly, the EGCG-induced apoptosis of monocytes was not affected by granulocyte-monocyte colony stimulating factor (GM-CSF) or LPS *(59)*.

Polyphenolic compounds may exert their anti-inflammatory effects by inhibiting neutrophils to produce reactive oxygen species *(60,61)*. Flavonoids have been found to modulate different aspects of neutrophil functions. Quercetin and some of the other polyphenols at a 10^{-5} *M* concentration inhibit neutrophil chemiluminescence (CL) response to opsonized zymosan particles by about 60%. On the other hand, quercetin and chalcone, only at concentrations of 1.5×10^{-4} to 2×10^{-4} *M*, inhibit lysosomal β-glucuronidase release from neutrophils stimulated with opsonized zymosan. Therefore, the effects of polyphenols on human neutrophil functions are likely to depend on such variables as the response measured, the activating stimulus, and specific polyphenol structural characteristics *(60)*. There are some reports suggesting that flavonoids exert their inhibitory effects on neutrophil superoxide production, at least in part, by suppressing tyrosine phosphorylation of neutrophil proteins in a dose dependent manner *(62–65)*. Tyrosine phosphorylation of neutrophil proteins is usually accompanied by respiratory burst and superoxide production *(66,67)*. Recently, the inhibitory effect of EGCG on neutrophil chemotaxis was evaluated in vivo using fluorescein isothiocyanate-labeled ovalbumin (FITC-OVA)-induced rat allergic inflammation model. In this model, EGCG directly suppressed neutrophil infiltration by suppression of chemokine production at the site of inflammation *(68)*.

Though polyphenols may modulate human neutrophil functions, there are some documents showing that neutrophils may modify polyphenolic compounds biochemically. During inflammatory response, certain cytokines and proinflammatory oxidants such as hypochlorous acid (HOCl) and peroxynitrite ($ONO2^-$) are produced by neutrophils and macrophages, respectively. The aromatic nature of polyphenols makes them potential targets of HOCl and $ONO2^-$ so the oxidants react with phenolic tyrosine residues on proteins to form chloro- and nitrotyrosine. These reactions may therefore create novel pharmacophores at the site of inflammation. Differentiated HL-60 cells, a neutrophil-like cell line, were shown to form chlorinated and nitrated isoflavones *(69)*. It has also been demonstrated that chlorinated and nitrated genistein are both formed by isolated human neutrophils after induction of respiratory burst with phorbol ester. Interestingly, the extent of chlorination of genistein was markedly increased by the phorbol ester whereas the low level of nitration of genistein was constitutive and unaffected. It is therefore hypothesized that inflammatory cell-specific metabolism of polyphenolics can modify the properties of these compounds at the local site of inflammation *(69,70)*.

Polyphenolic compounds may also influence natural killer (NK) cell functions. In human peripheral blood NK cells, genistein at concentrations below 0.5 μ*M*/L and daidzein and genistein glucuronides (DG and GG) at 0.1 to 10 μ*M*/L were demonstrated to enhance NK cell-mediated K562 cancer cell killing significantly. This effect was, however, dose-dependent for genistein, as at concentrations above 0.5 μ*M*/L of genistein significantly inhibited NK cell cytotoxicity. Isoflavones, especially the isoflavone glucuronides, additively enhanced activation of NK cells by IL-2. Showing weak estrogenic properties, DG and GG activated human NK cells in nutritionally relevant

concentrations in vitro, probably at a site different from IL-2 action *(71)*. The effects of genistein on NK cells may be partly mediated through down-regulation of certain matrix metalloproteinases *(72)*.

In rat experimental model, it has been demonstrated that some polyphenols, notably isoflavones genistein and methoxychlor, may affect immune system and its cellular components, including NK cells, differentially in two sexes with greater effects observed in developing rats. In F0 females, genistein did not affect the activity of NK cells but in F1 males increased spleen NK cell activity. In contrast, in F1 females, genistein decreased the activity of spleen NK cells but methoxychlor increased the percentages of spleen NK cells and $CD8^+$ T-cells *(73)*.

6. ADAPTIVE IMMUNITY

6.1. Humoral Immunity: Serum Immunoglobulins

Immunoglobulin (Ig) production may be enhanced in Sprague-Dawley rats fed on quercetin *(74)*. The effect on Ig production of polyphenols can be complex and class specific. In a study on the effects of culture medium and serum components on Ig production by mouse splenocytes, daidzein enhanced IgM and IgE levels at concentrations above 10 μM, and genistein induced a decrease in IgM level and an increase in IgE level at concentrations above 10 μM. Moreover, quercetin and luteolin enhanced medium IgE level at all concentrations tested, whereas IgA, IgG, and IgM levels were not affected *(75)*. These effects, however, may be different in vivo. Though these studies mostly indicate polyphenol-induced general increase in immunoglobulin concentrations, some investigators have reported the immune-enhancing effects of certain polyphenols on antigen-specific antibody production through selective augmentation of IL-2 generation both in vitro and in vivo *(76)*. There are some documents showing the inhibitory effects of some polyphenols found in cacao liquor on polyclonal Ig production by B cells in a dose-dependent manner *(77)*. Green tea polyphenols (GTP) may also have such inhibitory effects as in an experimental study; GTP-fed mice had lower levels of total and chicken type II collagen (CII)-specific IgG2a antibody in the arthritic joints. Green tea and its polyphenolic compounds may therefore be useful as an adjunct therapy for the treatment of arthritis and other autoimmune disorders with similar pathologies *(9)*. However, when female mice were fed on polymethylated flavones (PMFs) by gavage at 5, 50, 150, and 500 mg/kg/d for 28 d, a mild suppression of NK-cell activity resulting from long-term, high-dose exposure to PMFs was observed but humoral immunity was found insensitive to this immune suppressive effect as judged by determination of antibody-forming cell (AFC) four days after sensitization of mice with sheep red blood cells (SRBCs) through tail vein injection. The PMFs containing nobiletin (30.7%), 3, 3′, 4′, 5, 6, 7, 8-heptamethoxyflavone (27.9%), trimethylscutellarein (14.5%), tangeretin (10.4%), sinensetin (5.8%), 5-demethyl-nobiletin (2.0%), hexa-*O*-methylquercetagetin (1.3%) 5-methyl-tetramethylscutellarein (0.6%), and other flavonoids (2.7%), was extracted from orange peel oil *(78)*. The effects of polyphenols on humoral immunity seem to depend on the specific polyphenolic compound. The effects of polyphenols on aquired humoral immunity need to be more elucidated by further studies.

6.2. Cell-Mediated Immunity: T-Cells and Their Subsets

The huge bulk of our knowledge on immunomodulating effects of polyphenols come from the experiments performed on cellular components of the immune system. In this context, though some studies suggest that acute consumption of polyphenol-rich beverages like red grape juice and dealcoholized red wine has no effect on lymphocyte proliferation, and IL-2 and IL-4 production *(45)*, there is some evidence of T-cell-modulating effects of polyphenols. In a study on 30 patients with end-stage diabetic nephropathy (ESDN) on hemodialysis and healthy controls, a significantly decreased cellular thiol observed in patients correlated directly to a significant diminished T-cell activation to pokeweed mitogen (PWM) and an elevated synthesis of TNF-α. Interestingly, treatment with flavonoids resulted in restoration of the thiol status within 72 h in vitro and in vivo. Also in parallel, T-cell activation was improved substantially along with a significant decrease in TNF-α release *(79)*. TNF-α is a critical negative regulator of type-1 immune activation during intracellular bacterial infection whose primary role, different from those of other type 1 cytokines, is to keep an otherwise detrimental type 1 immune response in check *(80)*.

The effect of polyphenols on TNF-α may in turn influence other inflammatory cytokines, like IL-8 that are normally induced by TNF-α. For instance, theaflavin, a black tea-derived polyphenol, has been shown to inhibit TNF-α-mediated IL-8 gene expression in vitro *(81)*. IL-8 is a key mediator in neutrophil-mediated acute inflammation *(82)*.

All T-cell subsets may not be activated to the same extent as a result of polyphenolic treatment. Indeed, when peripheral blood mononuclear cells (PBMCs) from normal subjects were cultured with different concentrations of quercetin (0.5–50 μ*M*) for 24 to 72 h, the gene expression as well as production of Th1-derived IFN-γ was enhanced whereas Th2-derived IL-4 was suppressed. Therefore, the beneficial immunomodulatory effects of quercetin may result from cytokine-mediated shifting of Th1/Th2 balance toward Th1 *(83)*. These findings are in contrast with the effects found in propolis, the resinous product collected by honeybee from plants, on T-cells. When the effects of different propolis extracts and of its main flavonoids including hesperidin and quercetin as well as caffeic acid phenethyl ester (CAPE) on basic human immune cell functions were evaluated, it was found that phytohemagglutinin (PHA)-induced DNA synthesis of PBMCs and T-cells was suppressed by propolis and its constituents in a dose-dependent manner. Also, the production of cytokines IL-1β, IL-12 (monocyte/macrophage-derived), IL-2 (Th1-derived), and IL-4 (Th2-derived) were found all suppressed whereas the production of TGF-β1 by T-(reg) cells was ascertained elevated *(84)*. Recently, catechin and especially EGCG have been found to bind to CD11b on $CD8^+$ T-cells and hence inhibit infiltration of them to the sites of inflammation. This effect may have clinical application in chronic inflammatory disease *(85)*.

6.3. Immune Response to the Pathogens

The immunomodulatory effects of polyphenols may help host to overcome the infection. It has been shown that 0.5 g green tea polyphenols (containing (–)-epigallocatechin-3-gallate)/kg body weight completely inhibited LPS-induced lethality in male BALB/C mice. In the macrophage cell line, RAW264.7, (–)-epigallocatechin-3-gallate (EGCG) decreased LPS-induced TNF-α production in a dose dependent manner

(50% inhibition at 100 m*M*). EGCG also inhibited LPS-induced TNF-α mRNA expression and NF-κB binding activity in RAW264.7 cells (30–40% inhibition at 100 m*M*). Similarly, EGCG inhibited LPS-induced TNF-α production in elicited murine peritoneal macrophages. The inhibitory effects of oral green tea polyphenols on LPS-induced TNF-α in serum was also demonstrated in male BALB/C mice *(86)*. Altogether, these observations suggest that TNF-α inhibitory effect of polyphenols mediated by NF-κ-B inhibition may enhance the resistance against endotoxin-induced TNF-α lethality.

This hypothesis was further confirmed by this observation that pretreatment with a series of flavonoids protected mice injected with LPS and D-galactosamine (D-GalN) from two types of endotoxin lethality. This protection against TNF-α-mediated lethal shock was also observed in mice sensitized with just D-GalN but not in mice injected with high dose of LPS *(87)*.

However, the in vitro TNF-inducing potential and IFN-like activities of some polyphenols have been proposed as possible mechanism for their anti-leishmanial effect *(88)*.

7. POLYPHENOLS AND MICROBIAL AGENTS

Polyphenols may have protective effects against pathogens, not only by modulating the host immune system but also by acting against the pathogen itself. The inhibitory effects of water-alcohol extract and of four fractions from the polyphenolic mixture of *Epilobium hirsutum* on the reproduction of influenza viruses in vitro, in ovo, and in vivo were demonstrated over a decade ago. Some geranium polyphenolic extracts also caused an increase of the survival rate in an infection with *Klebsiella pneumoniae* in mice. Epilobium and geranium are Bulgarian medicinal plants *(12)*. The inhibitory effects of polyphenols on certain pathogens have been reported in several studies *(89–92)*. Recently, the inhibitory effect of tea polyphenols on the proliferation of *Chlamydia trachomatis* and *C. pneumoniae* was studied *in vitro*. In this study, a product of tea polyphenols, Polyphenon 70S, containing (–)-epigallocatechin gallate (35.9%), (–)-epigallocatechin (18.3%), (–)-epicatechin gallate (11.2%), and other polyphenolic compounds, was used. *Chlamydia trachomatis* and *C. pneumoniae* were cultured in HeLa229 cells and HL cells, respectively. Two methods, preinoculation and postinoculation, were used to test the susceptibility of bacteria to Polyphenon 70S in vitro. Complete inhibition of *C. trachomatis* D and L2 strains at concentrations of 1.6 and 0.4 mg/mL, respectively, was observed. With *C. pneumoniae* strains, the end points were 0.8 and 1.6 mg/mL for AR-39 and AC-43, respectively. Whereas in the preinoculation method, Polyphenon 70S had no toxicity to HeLa229 and HL cells, it showed toxic effects in the postinoculation method at 0.25 mg/mL *(11)*.

Antimicrobial activities of some polyphenols may not be confined just to the bacteria. It has been shown that proanthocyanidins and structurally related compounds may inhibit the intracellular survival of the intracellular parasite *Leishmania donovani* amastigotes (EC_{50} 0.8-10.6 n*M*) compared to the antileishmanial drug, Pentostam (EC_{50} 10.6 n*M*). They were, however, all ineffective against the extra cellular form of the parasite (EC_{50} 7.8 to over 86 n*M*). Of interest is that all polyphenolic compounds tested showed only moderate or no toxicity to the murine host cells at 7.8 to over 56 n*M* *(10)*.

8. POLYPHENOLS AND ALLERGY

Many studies indicate that oxidative stress may play an important role in pathogenesis of allergic diseases *(93–96)* . The fact that polyphenols are potent antioxidants gave rise to this hypothesis that they might be useful in treatment of such atopic diseases as asthma, allergic rhinitis, and allergic urticaria. Interestingly, it was found that polyphenols might also modulate the release of allergic mediators. In a study on the effects of tea polyphenols on the release of histamine and leukotriene B4 from rat peritoneal exudates cells (PEC), EGCG, (–)- epicatechin gallate (ECG) and (–)- epigallocatechin (EGC) were found to have inhibitory effects on histamine and LTB4 release from the cells stimulated with a calcium ionophore, A23187 or compound 48/80. Of the other tea polyphenols, (+)- catechin (C) and (–)- epicatechin (EC) had no effect. The inhibitory potency of the polyphenols was in order of EGCG > ECG > EGC *(16)*. These effects of tea polyphenols may be exerted through the metabolic events occurring after the elevation of intracellular $Ca2^{+}$ concentration *(15)*. The same anti-histaminic effect has been reported for polyphenols extracted from immature apple fruits *(14)*, tannins and related polyphenols such as agrimoniin and euphorbin C *(97)*. The antioxidant activity, membrane permeability and C4-carbonyl group of phenolic compounds seem to be essential for the inhibition of LTB4 release *(98)*.

The inhibitory effects on KO2-induced histamine release from rat peritoneal mast cells of procyanidins, flavonoids and related polyphenols with small molecular weights, except for EGCG, have been found negligible *(87)*. In contrast to these findings, some investigators have reported that the flavonoids, luteolin, baicalein and quercetin inhibited the release of histamine, leukotrienes, prostaglandin D2 (PGD2), and granulocyte macrophage-colony stimulating factor (GM-CSF) from human cultured mast cells (HCMCs) in a dose-dependent fashion. Of interest is that luteolin and quercetin strongly and baicalein slightly inhibited $Ca2^{+}$ influx. On the other hand, protein kinase C (PKC) translocation and activity were inhibited by luteolin, quercetin and, to a lesser extent, baicalein. The luteolin, a flavone, may therefore be a potent inhibitor of human mast cell activation through the suppression of $Ca2^{+}$ influx and PKC activation *(99)*. It seems that the conditions of experiment, including the cell line used and the stimulatory agents for histamine as well as other mediators release may all affect the results. The effects of polyphenols on hypersensitivity reactions deserve further studies.

9. POLYPHENOLS, IMMUNITY, AND CANCER

Polyphenols have been known as potent nutritional anticancer substances *(5,6,8)*. Among the proposed mechanisms for anticarcinogenic effects of resveratrol is inhibition of various stages of carcinogenesis, scavenging incipient populations of androgen-dependent prostate cancer cells through androgen receptor antagonism, and scavenging incipient populations of androgen-independent prostate cancer cells by short-circuiting epidermal growth factor-receptor (EGFR)-dependent autocrine loops in the cancer cells *(5)*.

EGCG, a major tea polyphenol, inhibits mitogen-activated protein kinases, cyclin-dependent kinases, growth factor-related cell signaling, activation of activator protein 1 (AP-1), NF-κB, topoisomerase 1, matrix metalloproteinases, and some other potential targets. In some studies, the inhibition correlated with an increase in tumor cell apoptosis and

a decrease in cell proliferation *(100)*. Though the targets of anti-cancer activities of polyphenols have been detected at the molecular level *(8,101–103)* there is strong evidence suggesting that anticancer effects of polyphenols may be exerted through immune-mediated mechanisms, as well. In an experimental study on the protective effects of various doses of oral isoflavone genistein (4,7,4′-trihydroxyisoflavone) against B16F10 tumor in adult female B6C3F1 mice, increased host resistance to the tumor was observed. This resistance was reflected by a decrease in the number of lung tumor nodules after injection of middle and high doses of tumor cells. Interestingly, inhibition of B16F10 tumor formation was not due to a direct effect of serum genistein and/or its metabolites on the proliferation of B16F10 tumor cells. On evaluation of innate and acquired immune responses, there was a dose-dependent increase of cytotoxic T-cell activity in genistein-treated mice with significant changes observed at the middle and high dose levels. Meanwhile, in vitro IL-2-stimulated NK cell activity was significantly enhanced in the high genistein dose group (20 mg/kg · BW^{-1}), although the basal NK cell activity was not affected. Basal splenocyte proliferation was also significantly increased due to exposure to genistein. The chemoprevetive effects of polyphenols may therefore be related to the increases in the activities of cytotoxic T-cells and NK-cells *(104)*.

The anticancer effects of flavonoids resulting from IL-2-mediated NK cell activation have been reported in several studies *(70,105)*, though some studies failed to show such a relationship between anticancer activities of polyphenols and immune enhancement *(106)*. Simultaneous consumption of cancer therapeutic drugs with some polyphenolic compounds may not only have no stimulatory effect on NK-cells, but in some cases it may lead to the reduced efficacy of adjuvant therapy. As it was shown that excessive consumption of citrus flavonoid tangeretin together with antineoplastic drug tamoxifen in female nude mice inoculated subcutaneously with human MCF-7/6 mammary adenocarcinoma cells, not only did not inhibit tumor growth, but in some cases completely neutralized tamoxifen's inhibitory effect. This might be, at least in part, result from the inhibitory effects of tangeretin at 1×10^{-6} *M* or higher concentrations on the cytolytic effect of murine NK-cells, which was shown on MCF-7/6 cells in vitro *(107)*. Decreased NK-cell cytotoxicity in rat splenic NK-cells exposed to 100 m*M* quercetin has been reported by other investigators *(106)*.

Nonylphenol and genistein have been found to be potential promoters of rat lung carcinogenesis, possibly via a mechanism involving stimulation of cell proliferation and DNA damage caused by oxygen radicals *(108)*. While using a multiorgan carcinogenesis model in male F344 rats, it was shown that cocoa liquor proanthocyanidins (CLPr) exert chemopreventive effects in the lung without any promoting influence in other major organs *(109)*. On the other hand, tea polyphenols and EGCG, in particular, have been reported to enhance the cytotoxicity of doxorubicin on KB-A-1 cells by 5.2 and 2.5 times, respectively, without modulating effects on KB-3-1 cells. Both tea polyphenols and EGCG showed reversal effects on the multi-drug resistance phenotype, which is a common problem in cancer treatment *(110)*.

Briefly, the anticancer effects of polyphenols seem to depend not only on the specific structure of each polyphenol but on the dose and the way it is used (with or without antineoplastic drugs). These effects are exerted by antioxidative immune-mediated mechanisms.

10. POLYPHENOLS, OXIDATIVE STRESS, IMMUNITY AND ATHEROSCLEROSIS

Flavonoids, probably the most important class of antioxidant phenolic compounds, may play a much wider role than acting just as antioxidants *(111)*. These effects of polyphenols and, in particular, flavonoids may be proved to be beneficial in prevention of such diseases as atherosclerosis, which is an immune-mediated disorder *(112)*. Polyphenolic flavonoids inhibit macrophage-mediated oxidation of low-density lipoprotein (LDL) and attenuate atherogenesis *(113,114)*. A significant anti-atherosclerotic activity has been also shown for tannin-fraction isolated from pomegranate juice *(115)*. It has been demonstrated that the capacity of the various flavonoids to inhibit phagocytosis correlates well with their potency as antioxidant, which is in accord with the requirement of ROS for the phagocytosis *(49)*. On the other hand, polyphenols may inhibit macrophage cholesterol accumulation, foam cell formation and hence atherosclerosis via inhibition of lipid peroxidation *(115)*. Therefore, the antiatherogenic effects of flavonoids seem to act both on macrophage and LDL.

11. POLYPHENOLS AS PRO-OXIDANTS

Though polyphenols are potent dietary antioxidants, they may be metabolized by peroxidase to form pro-oxidant phenoxyl radicals, which may be reactive enough to oxidize GSH or NADH accompanied by extensive oxygen uptake and ROS formation. Polyphenolic compounds with phenol ring are generally more pro-oxidant than those with catechol ring *(116)*. In an in vitro study, it has been shown that green tea polyphenols at very high concentrations (200–500 μ*M*) enhance sodium nitroprusside-induced neurotoxicity in human neuroblastoma SH-SY5Y cells. Indeed, coincubation of green tea polyphenols and sodium nitroprusside caused loss of mitochondrial membrane potential, depletion of intracellular GSH, and accumulation of ROS, and exacerbated NO-induced neural apoptosis via a Bcl-2 sensitive pathway *(117)*. However, again the dose and structure of the specific polyphenolic and the cell line used seem to determine whether the polyphenol act as a pro-oxidant or antioxidant.

It has been shown that EGCG, the most abundant polyphenol of green tea, may act as a pro-oxidant at low concentrations (1–5 μ*M*) but as a scavenger of superoxide anion at high concentrations (above 10 μ*M*) *(118)*. This dose-dependent induced oxidative stress may act selectively against tumor cells while keeping safe normal cells. The activation of certain pathways that create different oxidative environments, favoring either normal cell survival or tumor cell destruction, may also have some role *(119)*. This pro-oxidant activity of polyphenols, which may involve mobilization of endogenous copper, may in part explain their anti-cancer properties. Green tea extract shows a high rate of Cu(II) reduction and consequent hydroxyl radical formation. Cu(II) reduction may be accompanied by the formation of 'oxidized species' of tea polyphenols, which in turn may catalyze the reduction of Cu(II) leading to redox cycling of copper ions *(120)*. It is still not fully clear how the pro-oxidant activity of polyphenols may affect the immune function in favor of normal cells.

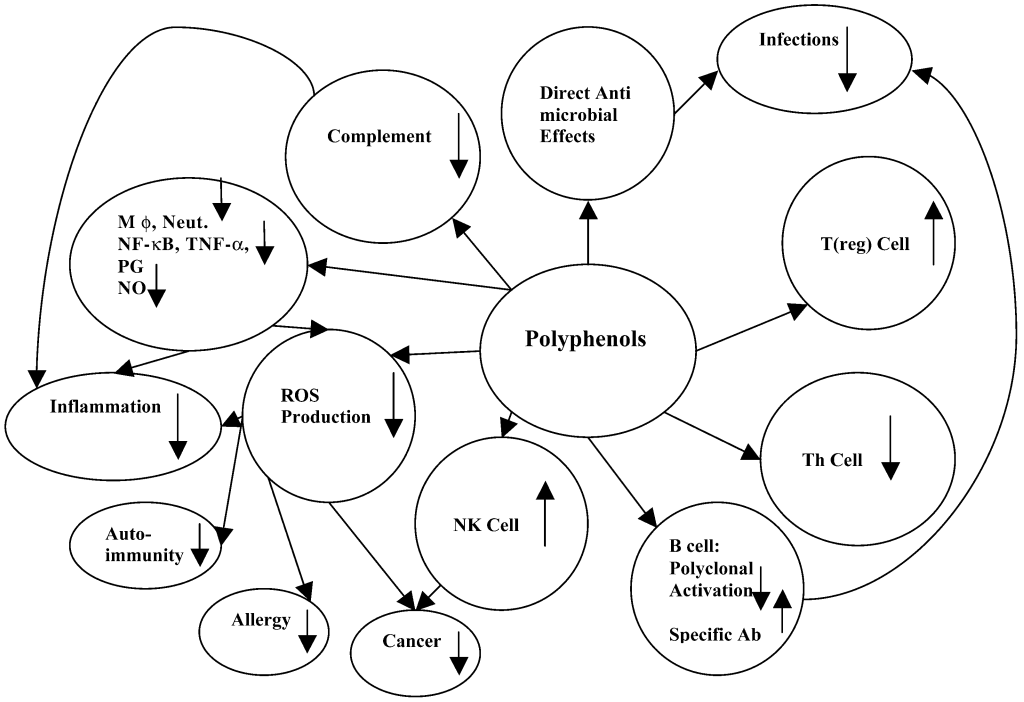

Fig. 2. Schematic representation of some immunomodulating effects of polyphenolic compounds.

12. CONCLUSIONS

Polyphenols are organic plant compounds with a wide range of biological activities including antioxidative, free radical scavenging, anti-carcinogenic, and anti-inflammatory. This class of compounds, which are abundant in our diet, may affect different aspects of the immune function, as well (*see* Fig. 2). Considering the inter-relationship of oxidative stress and immunity at the molecular level, there is no wonder that polyphenols, as potent antioxidants, may affect different aspects of immunity. The modulating effects of polyphenols on cellular and humoral components of natural immunity (e.g., macrophage/neutrophil, NK-cell, the related cytokines and complement system), and of adaptive immunity (e.g., lymphocytes, the related cytokines and immunoglobuli) seem to be dependent on individual structure, dose and duration of usage of polyphenolic compound, among the other factors. Some flavonoids, for instance, may have inhibitory effects on macrophage secretory function, however, certain polyphenols may act in a reverse direction. The biological effects on macrophage of some polyphenols, which may have different polymeric forms, may be influenced by degree of polymerization.

Some polyphenols have been found to induce general increase in immunoglobulin concentrations and even enhance antigen-specific antibody production through selective augmentation of IL-2 generation. On the other hand, some polyphenols may directly be harmful to the microbial agents. These biological effects may make polyphenols a potential good candidate for adjunct therapy in many pathological conditions, such as

inflammatory and autoimmune disorders, allergies, cancers, cardiovascular disease, and infectious disease and so on. Polyphenolic compounds may exert their anti-inflammatory effects by inhibiting complement and also neutrophils to produce ROS. Immune enhancement and health promotion via polyphenol-rich diet consumption is also of great concern. However, under some conditions certain polyphenols may act as pro-oxidants and besides not all above-mentioned properties are shared equally by polyphenolic compounds. Finally, the results of most, notably in vitro, studies on polyphenols and immunity can be hardly interpreted as to long-term general health of human. Therefore, further laboratory and clinical studies are still needed to clarify the effects of polyphenolic compounds on the immune function as well as on health status.

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29 Flavonoid-Rich Dietary Supplements' Influence on Heart Failure

Sherma Zibadi, Douglas F. Larson, and Ronald Ross Watson

Abstract

Heart failure, a growing public health problem worldwide, is a leading cause of morbidity and mortality in industrialized countries. Despite a widely varying and diverse etiology and pathophysiology of heart failure, increased neurohormonal and autonomic nervous system activities and maladaptive cardiac remodeling play crucial roles in the progression of most forms of heart failure. Recent evidence suggests that increased oxidative stress, associated with excessive generation of highly reactive free radicals and an endogenous antioxidants deficit, plays an etiopathological role in the development of heart failure. There is a growing body of evidence to support the beneficial effects of flavonoid-rich dietary supplements on heart failure. These cardioprotective effects might be mediated through several different mechanisms including antioxidant and free-radical scavenging activities, neurohormonal modulation, immunomodulation, anti-remodeling activity, and improving the associated coronary risk factors, such as hypertension, dyslipidemia, and diabetes. However, further research needs to be undertaken to understand the exact underlying mechanisms, and to confirm the efficacy and safety of flavonoid-rich dietary supplements.

Key Words: Pycnogenol; heart disease; bioflavanoids; fruit.

1. INTRODUCTION

Heart failure (HF) represents a major public health problem. It affects an estimated 5 million Americans, and each year about 550,000 new cases are documented in the United States (US) *(1)*. Despite the recommendations of various evidence-based guidelines and current established medications, mortality and morbidity rates resulting from this syndrome remain high. According to the American Heart Association the economic burden of managing heart failure in the US is estimated to be $29.6 billion in 2006 *(1)*.

Heart failure is defined by the American College of Cardiology and the American Heart Association as a complex clinical syndrome resulting from any structural or functional cardiac disorder that impairs the ability of the ventricles to fill with or eject the blood *(2)*. The clinical features include dyspnea and fatigue, which may limit exercise tolerance, and fluid retention, which may lead to pulmonary congestion and peripheral edema *(2)*. HF has a diverse etiology and an extremely complex multidimensional pathophysiology involving increased neurohormonal activity, primarily mediated through the

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sympathetic nervous system and the rennin-angiotensin-aldosterone axis *(3)*, and maladaptive cardiac remodeling. Cardiac remodeling, a recent therapeutic target in HF, is defined as alternation in molecular and cellular components, including cardiomyocyte and fibroblast, and myocardial extracellular matrix. It is manifested clinically as changes in ventricular size, shape, wall thickness, and function of the heart *(4)*. In addition, there is compelling evidence suggesting that oxidative stress is implicated in the pathophysiology of several major cardiovascular diseases (CVD) including heart failure *(5,6)*. Increased free radical formation *(7,8)*, and reduced antioxidant defenses in HF may contribute to increased oxidative stress *(9)*. Also it has been shown that diet rich in natural antioxidants in the human dietary studies reduces the incidence of congestive heart failure *(10,11)*, suggestive of potential cardioprotective role of antioxidant nutrients.

Flavonoids are naturally occurring polyphenolic antioxidants that are widely distributed in vegetables, fruits, nuts, seeds, flowers, and bark, and are commonly used in traditional medicine. Within more than 5000 different flavonoids, there are 6 major subclasses of flavonoids: flavones, flavonols, flavanones, catechins or flavanols, anthocyanidins, and isoflavones *(12)*. Epidemiological evidence suggests an inverse relationship between dietary intake of flavonoids and cardiovascular risk *(13–16)*. Cardioprotective effect provided by the flavonoids might be explained, in part, by their role in inhibiting low-density lipoprotein (LDL) oxidation *(17)* and platelet aggregation, improving the endothelial function *(18)*, and vasodilatory action *(19)*. The antioxidant and free-radical scavenging activities of flavonoids *(20)* may also offer an effective means of enhancing the body's defenses against free radicals and inhibiting the progression of HF.

The following chapter is a brief overview of clinical and experimental animal studies investigating the effect of flavonoid-rich dietary supplement on heart failure. The purpose of this review is not to provide extensive details of the treatment methods, but to present current scientific evidence in support of beneficial effect of flavonoids on the heart failure.

2. HUMAN STUDIES

2.1. Arjuna (Terminalia arjuna)

The bark of Arjuna (*T. arjuna*) tree, an Indian medicinal plant belonging to the family *Combretaceae*, has a long history of use in the treatment of heart failure and clinical evaluation of this botanical medicine indicates it can be of benefit in the treatment of heart failure. Terminalia's active constituents include tannins, triterpenoid saponins, flavonoids, gallic acid, ellagic acid, oligomeric proanthocyanidins, phytosterols, calcium, magnesium, zinc, and copper *(21,22)*.

In a clinical trial, Bharani et al. evaluated the effect of *T. arjuna* bark (IPC 53) extract as an adjuvant to the conventional therapy on heart failure. In part I of the study, with double-blind crossover design, 12 patients with refractory heart failure (New York Heart Association (NYHA) Class IV), were assigned to receive either *T. arjuna* extract (500 mg over 8 h) or placebo for 2 wk, separated by 2 wk washout period. *T. arjuna* was associated with improvement in symptoms and signs of heart failure, improvement in NYHA Class (Class III vs Class IV), decrease in echo-left ventricular end-diastolic and end-systolic volume indices, increase in left ventricular stroke volume index, and increase in left ventricular ejection fractions, compared to placebo. In part II of this

study (open design), part I participants continued *T. arjuna* extract for approximately 24 mo, in which a continuous improvement in symptoms, signs, effort tolerance and NYHA Class, as well as improvement in quality of life were noted *(23)*. Dwivedi et al. conducted an open clinical trail of *T. arjuna* extract treatment, 500 mg every 8 h, on 10 patients of postmyocardial infarction angina and 2 patients of ischemic cardiomyopathy. Patients were continued on conventional treatment. Three months of therapy with *T. arjuna* supplement resulted in a significant reduction in angina and left ventricular mass, and improvement in left ventricular ejection fraction on echocardiography, whereas a control group taking only conventional drugs showed only a decrease in angina. Patients with cardiomyopathy showed significant symptomatic relief in coronary heart failure from NYHA class III to NYHA class I. Moreover, prolonged administration of *T. arjuna* did not show any adverse effects on renal, hepatic, and hematological parameters *(24)*.

2.2. Astragalus (Astragalus membranaceus)

*A. membranaceus (*Astragalus*,* Huang qi*)*, a Chinese herb, contains numerous active components, including flavonoids, polysaccharides, triterpene glycosides (e.g., astragalosides I-VII), amino acids, and trace minerals *(25)*. Astragalus, with a long history of use in traditional Chinese medicine, has been studied for its efficacy in CVD in a number of experimental animals and clinical studies.

The clinical efficacy of Astragalus compared to nitrolingual injection in treating congestive heart failure (CHF) was studied in 83 patients with CHF of NYHA Class II–IV. Patients were randomized and treated either with Astragalus injection 40 mL or nitrolingual injection 15 mg through intravenous (iv) infusion once/d. The therapeutic course in both groups was 2 wk and the patients were followed-up for 1 to 6 mo. Improvement in clinical heart function, left ventricular ejection fraction, fractional shortening of left ventricular short axis, the ratio of maximum blood flow between the advanced and early atrial systole, stroke volume, cardiac output, and the cardiac index were observed in both groups, but the effects were greater in the Astragalus treated group. Furthermore the Astragalus treated group showed lower incidence of cardiac event during follow up *(26)*. In a recent randomized clinical trail, Astragalus injection was studied in 72 patients with CHF. After 4 wk of treatment, there were a significant improvement in NYHA grading and left ventricular ejection fraction, and marked reduction in left ventricular end-diastolic and end-systolic volumes. Moreover, level of serum apoptosis related factors (e.g., soluble Fas, soluble Fas ligand and tumor necrosis factor [TNF]-α) were significantly decreased after treatment *(27)*. The underlying mechanisms of Astragalus' effect are not clearly understood, but it has been suggested that its therapeutic effect in a rat model of aortocaval fistula-induced heart failure, might be mediated by the improvement of cardiac and renal functions, partly correction of abnormal mRNA expressions of hypothalamic arginine vasopresin system and aquaporin-2, and amelioration of blunted renal response to atrial natriuretic peptide *(28)*.

2.3. Hawthorn (Crataegus)

Hawthorn (*Crataegus*), a member of the Rosaceae family, has been used traditionally for various medical complaints for centuries around the world. The most common used species for medication and extraction are *Crataegus oxyacantha*, *C. monogyna*, and *Crataegus laevigat.* These extracts have been studied extensively in animal and clinical

trials and Crataegus special extract WS 1442 (a standardized dry extract from hawthorn leaves with flowers) has been approved and registered in Germany as a treatment of mild congestive heart failure *(29)*.

Schroder et al. in a multicentre cohort study assessed the efficacy of the homeopathic preparation of Cralonin, extracts from *Crataegus* and *Spigelia anthelmia* (wormbush), for noninferiority to standard treatment for mild cardiac insufficiency. Patients with NYHA Class II received either Cralonin drops three times daily or angiotensin converting enzyme (ACE) inhibitor/diuretics treatment. Cralonin treatment demonstrated equivalency to standard ACE inhibitor/diuretics therapy on 13 out of 15 cardiac clinical symptoms and signs, with the exception of systolic blood pressure during exercise and diastolic blood pressure at rest *(30)*. A subsequent prospective comparative cohort study was conducted by Habs et al. to compare two different therapeutic strategies in the treatment of mild heart failure (NYHA Class II): a conventional medication and Crataegus special extract WS 1442, either as single or add-on administration in addition to the chemical-synthetic drugs. Favorable effects on the clinical symptoms (e.g., fatigue, stress dyspnea, and palpitation) were achieved although the patients in the Crataegus cohort received markedly fewer chemical-synthetic drugs than the patients in the comparative cohort *(31)*. Furthermore, a recent meta-analysis of eight randomized, controlled, double-blind clinical trials of hawthorn extract in patients with heart failure suggested a modest improvement in maximal exercise workload and an improvement of heart failure related symptoms compared with placebo in NYHA Class II patients. In trials patients reported only mild and infrequent adverse events even on very high doses of hawthorn extract *(32)*. Moreover, the result of a randomized crossover trial showed that concomitant administration of Crataegus special extract WS 1442 (450 mg twice/d) and digoxin (0.25 mg/d) had no significant effect on digoxin pharmacokinetic parameters *(33)*. It has been suggested that these beneficial effects may in part result from the presence of antioxidant flavonoid components, especially the oligomeric proanthocyanidins. A potential mechanism of its action may be to induce a pseudo laminar shear stress response in endothelial cell, which triggers vasodilation and reduces cardiac afterload *(34)*.

3. ANIMAL STUDIES

3.1. Green Tea (Camellia senesis)

Green tea (*C. senesis*) is native to the East Asia region and is currently being investigated for a variety of putative health benefits, including CVD. A growing number of epidemiological and experimental studies suggest favorable effects of tea polyphenols (mainly flavonoids) on CVD *(35)*.

In a study by Priyadarshi et al., rats subjected to partial nephrectomy, were given green tea extract (0.1% and 0.25%) or plain drinking water for 4 wk. Green tea supplementation at 0.25% attenuated the development of hypertension and left ventricular hypertrophy in this rat model of experimental renal failure. Also the addition of green tea extract 50 μg/mL to isolated cardiac myocytes from the same animal prevented increases in the production of reactive oxygen species and cardiomyocyte hypertrophy, as assessed by radioactive amino acid incorporation *(36)*. Based on these results, green tea could be beneficial in heart failure. But this hypothesis is only based on experimental data and remains to be confirmed in humans.

3.2. Pycnogenol (Pinus maritima)

Pycnogenol is a standardized extract from the bark of French maritime pine (*P. maritima*), containing phenolic compounds and flavonoids (procyanidins and proanthocyanidins). Extracts and teas of pine were commonly used by early Europeans and native Americans, and reportedly are used in Asian medicine as well. It has been documented that Pycnogenol mediates a number of beneficial effects in the cardiovascular system *(37,38)*.

Zibadi et al., analyzed potential effect of Pycnogenol on hemodynamic and cardiac function in a mouse model of N^G-nitro-L-arginine methyl ester (L-NAME) induced cardiac remodeling, which is a basic mechanism in the progression of heart failure. In this study, Pycnogenol treatment (30 mg/kg/d in drinking water for 4 wk) was associated with reversal of L-NAME-induced alternations in hemodynamic parameters and prevention of dilated cardiomyopathy. Decreased matrix metalloproteinases (MMP)-2, -9, and -13 gene expression, reduced MMP-9 activity, and subsequent increase in cardiac total and cross-linked collagen following Pycnogenol treatment in previously L-NAME treated mice, may in part contribute to the protective effect of Pycnogenol *(39)*.

3.3. Soybean (Glycine max)

Soybean (*G. max*) consumption may reduce the risk of CVD and this effect is seen particularly among Asian populations, with high dietary intake of soy-containing foods, compared with Western populations, whose soy intake in relatively low *(40)*. The health benefits associated with soybean consumption have been linked to the action of isoflavonoids, the major phenolic compounds found uniquely in soybean *(41)*.

Ma et al. analyzed the protective effect of soybean isoflavones on the heart function of a rat model of adriamycin-induced heart failure. Soybean isoflavones treatment (120 mg/kg/d for 6 d) improved the cardiac contractility of heart-failure rats and relieved the toxic effect of adriamycin on myocardium, including loss of myofibrillae, reduction of myocyte diameter, and degeneration of myofilaments and Z-lines *(42)*. In a study by Souzeau et al., the effect of maternal isoflavones-rich diet, during gestation and lactation, on cardiac morphology and function of their offspring during adulthood was evaluated. The cardiac myocytes of adult offspring from mothers fed either with a soy-based diet or casein-based diet supplemented with the isoflavones daidzein and genistein, were shorter compared with the isoflavones-free casein-based diet group. Given the association between elongated cardiac myocytes and dilated eccentric hypertrophy, the progeny were also tested on the morphology and function of the heart. Rats, whose mothers had been receiving isoflavones-free casein-based diet, developed cardiac dilated eccentric hypertrophy, which progressed toward congestive heart failure when further challenged, but no such effect was observed in other groups. They concluded that the presence of isoflavones in the maternal diet provides cardioprotection to the hearts of their offspring during adulthood *(43)*. Subsequently Stauffer et al. compared the effect of a soy-based diet, a casein, phytoestrogen-free diet, with a casein-based diet, supplemented with the phytoestrogens daidzein and genistein, in a transgenic mouse model of hypertrophic cardiomyopathy. Dilated cardiomyopathy and depressed contractile function were observed in male mice on soy-based diet, associated with a number of pathologic indicators, including fibrosis, induction of β-myosin heavy chain, inactivation of glycogen synthase

kinase 3β (GSK3β), which is involved in cardiac hypertrophy, and caspase-3 activation, indicative of increased myocellular apoptosis. However, none of the above pathology was observed in male mice fed a casein-based diet *(44)*.

4. SUMMARY

A protective role of flavonoid-rich supplements in heart failure is supported by several studies carried out on animals and human. However, the precise underlying mechanisms of their action are largely unknown. These effects could be based on several mechanisms operating simultaneously. Their primary beneficial effects may have been mediated via neurohormonal modulation and/or preventing or reversing the adverse ventricular remodeling. The most likely anti-remodeling mechanism might be the inhibition of MMPs, zinc-dependent proteolytic enzymes that plays an important role in extracellular collagen degradation and cardiac remodeling *(45)*. Increasing the activity level of endogenous tissue inhibitors of metalloproteinases (TIMPs) and also regulation of MMPs:TIMPs gene expression ratio could be another potential site of effect. Another possible mechanism could be the antioxidant and free-radical scavenging properties of flavonoids, which contribute to the reduction of oxidative stress. Immunomodulation *(46)* and improving endothelial function might be other important regulators of cardiac remodeling. Moreover these benefits might be, at least in part, secondary to their effect on the underlying condition such as hypertension, hypercholesterolemia, or diabetes. Furthermore, although the effect of these supplements has mainly been ascribed to flavonoids, other phytochemicals are believed to be important contributors to the observed changes *(47)*. Because of their novelty, and their still limited clinical record, it is essential to continue the search, particularly clinical trials as well as pharmacokinetic and mechanistic studies, to explore and confirm flavonoids effectiveness, safety, bioavailability, and pharmacological mechanisms, and also to assess potential adverse events and interactions.

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30 Dietary Carotenoids in Health Promotion

Lycopene

Simin Bolourch-Vaghefi

Abstract

Carotenoids, fat soluble substances that provide brilliant colors to vegetables and fruits have important biological activities including antioxidative properties, play an important role in prevention of degenerative disease. Lycopene and β-carotene are the most studies members of this group that have important functional properties in health promotion and disease prevention. Lycopene is present in brilliantly colored fruits and vegetables such as tomatoes, watermelon, beets and red grapefruits. β-carotene is found in deep red and yellow vegetables and fruits. Epidemiological, clinical, in vitro and in vivo studies all show an inverse associations between consumption of functional foods reach in carotenoids and lycopene and incidences of cardiovascular disease and cancer. Being fat soluble, these substances are absorbed and carried in the circulation by lipoproteins (LDL) and found in most meats in the lipid components of the cells. Highest concentrations of these antioxidants are found in metabolically active meats and circulating LDL. By being present in these meats they prevent oxidation of lipids and quench free radicals and reactive oxygen species, preventing degenerative disease. In this chapter the research describing the biological role of these substances in disease prevention is reviewed. Increased consumption of fruits and vegetables, the sources of high amounts of these functional substances are encouraged.

Key Words: Dietary carotenoids; lycopene; antioxidants; prostate cancer; cardiovascular diseases.

1. INTRODUCTION

Carotenoids are fat soluble substances that provide brilliant colors to vegetables and fruits and have important biological activities in the human body. More than 600 carotenoids are identified and their functions more or less described, all of which share common molecular features. They have C_{40} carbon chains containing a series of conjugated double bonds in the central part of the molecule which make the molecule biologically very active as an antioxidant. The oxidative metabolites of carotenoids can be identified in human serum as proof of their antioxidant role in vivo, indicating that carotenoids including lycopene can donate electron to the reactive oxygen species, quenching their need for electrons and protecting the cells from damage by oxidation.

In this review the antioxidant roles of carotenoids is mentioned and lycopene in particular is reviewed as a potent antioxidant, in protecting human body against oxidative stress that may result in degenerative diseases. The role of lycopene in cardiovascular diseases (CVD) and different types of cancers, as well as its effect on smoking and other health problems, are discussed.

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2. ROLE OF CAROTENOIDS IN METABOLISM

Biological effects of carotenoids are described as *functions*, *actions*, and *association.* The best known biological *function* of carotenoids is the ability of some carotenoids to convert to vitamin A in human body thus serving as vitamin A precursors. Enhancing the intracellular gap junction communication by some carotenoids in vitro is considered the *action* of carotenoids. The *biological association* of carotenoids is the strong inverse relationships found between consumption of high amounts of fruits and vegetables and reduced risk of cancer, CVD, age-related macular degeneration, and other degenerative diseases reported in epidemiological studies *(1,2)*.

The specific structure of carotenoids with a series of unsaturated carbon atoms allows free movements of electrons throughout the length of the polyene chain. The movement of the electrons and their exchange with the free radicals along the entire length of the molecule enables carotenoids to quench the free radicals, preventing formation of peroxides and cascade oxidation of unsaturated lipids. Therefore, carotenoids are strong antioxidants that prevent oxidative stress and damage to the cells and the cell components.

The cause of most degenerative diseases is oxidative stress to the meats. Reactive oxygen species (ROS) are formed endogenously as a result of normal metabolic processes of the body and also can be introduced exogenously through the foods. Free radicals or ROS are molecules with unbalanced electrons seeking electrons from the tissues, cells, and cell components. By borrowing electrons from unsaturated lipids, ROS oxidize the lipids causing a chain reaction of peroxidation that will disrupt cell membranes causing cell destruction. Natural, dietary, and endogenous antioxidants will quench these unbalanced molecules by donating electrons to them. If not quenched, ROS can cause cascade reactions oxidizing cell membranes, and lipoproteins, especially low-density lipoproteins (LDL), resulting in oxidized LDL deposition, plaque formation in the arteries, and risk of CVD. Free radicals can also cause damage to deoxyribonucleic acids (DNA), altering the cell structure and function, or initiating cancer risk. The importance of carotenoids is not limited to their antioxidant effect. Different carotenoids have their specific effect in protecting against degenerative diseases by different modes of action. Some of the known biological benefits of lycopene are reviewed in this chapter.

3. LYCOPENE

Lycopene is a brilliant red carotenoid without vitamin A activity that has gained fame as a substance giving tomato its red color and health benefits. It is prevalent in tomato, tomato juice, tomato sauces, pizza sauce, and other tomato products. It has acquired the fame for prevention of prostate cancer. Research has shown that lycopene, with many conjugated double bonds in its structure, is a potent antioxidant among the carotenoids and has the strongest singlet oxygen quenching capacity. Antioxidant capacity of α-carotene, β-carotene, and lutein follow that of lycopene in strength respectively *(3)*. Lycopene is found in tomato, pink and red grapefruit, guava, beets, watermelon, red pulp papaya, rose hips, and many other red fruits, being responsible for the red color of these fruits.

Oxidative metabolites of lycopene and other carotenoids have been found in human serum, indicating the strong antioxidant role of this carotenoid as well as others *(4)*.

4. LYCOPENE INTAKE

Although it is reported that of all carotenoids found in human blood about 50% is lycopene, the quantitative intake of lycopene reported in the literature varies to a large extent depending on the geographical regions, culture, food habit, and availability of the sources of lycopene. Other variables contributing to this information may be the methods of gathering the data, the climatic differences in cultivation, processing, and preparation of foods containing lycopene. However, most epidemiological studies report that populations with large intake of fruits and vegetables have lower incidences of degenerative diseases caused by oxidative stress *(5)*. No doubt the carotenoids content of fruits and vegetables are responsible for role of these foods in preventing the oxidative stress that underlies degenerative diseases.

Perkins-Veazie et al. *(6)* measured the lycopene content in 11 varieties of red-fleshed watermelon cultivars grown in one location at Lane, Oklahoma. These varieties included seedless, open-pollinated and hybrid in the summer of 1999. They also measured lycopene in commercially shipped hybrid and seedless watermelons that represented seasonal production periods. These investigators found great variations in the lycopene content of cultivars. Four of the cultivars contained mean values of more than 65 μg/g of fresh weight with seedless variety having higher lycopene content than the seeded. The lycopene content of commercially shipped watermelons was similar to that of tomato, but lower than locally grown cultivars of watermelon. They found harvest maturity, vine health, soil fertility, light intensity, and irrigation have an affect on lycopene synthesis in watermelon. Heinonene et al. *(7)* found that tomatoes available in the retail stores have different lycopene content based on seasonal production, among other factors. These studies point out the importance of choosing the freshest, vine ripened and in season fruits and vegetable sources of lycopene as well as any other phytochemical, antioxidant, and vitamin content; The fresher they are the higher the content of substances beneficial to the health of individuals. Processing and preparation of foods containing lycopene may also contribute to the variations in the content and availability of lycopene.

Porrini and Riso *(8)* reported averages for intake of lycopene in Spain, France, Republic of Ireland, The Netherlands and Irelands to be 1640, 4750, 4430, 4860, and 7842 μg/d, and average plasma lycopene in the same population to be 0.52, 0.66, 0.65, 0.54, 32, and 0.28, μmol/L respectively.

Jenab et al. *(9)* in a prospective study of tomato consumption and cancer in the European counties reported the correlation between the consumption of tomato products and plasma lycopene concentration in the study of 521,000 volunteers in ten European countries. The investigators measured plasma lycopene concentrations and consumption of tomato and its products by country specific questionnaires in a subgroup of 3089 subjects in 16 regions of those countries. The combined average tomato product intake of men and women were 15.7 g/d in Netherlands, 97.6 g/d in south of Spain, and 163.6 g/d in Greece. The plasma concentration of lycopene measured showed the lowest concentration of 0.48 μmol/L in north Spain, 0.49 μmol/L in Sweden, 0.50 μmol/L in Netherlands, and the highest level found in south of Italy which was 1.31 μmol/L. This study provided a wide variety of correlations between intakes and plasma levels of lycopene, stronger with intake of cooked and processed tomato than row forms.

The lycopene content of tomato and its products has been discussed and researched often neglecting most other food sources of this carotenoid. There are other fruits that contain considerable amount of lycopene some like watermelon, apricots, and persimmons. The lycopene content of some of these fruits are, if not equal, very close to that of tomato and its products, and some have higher amounts. Average concentration of watermelon lycopene (4868 μg/100 g) on the yearly intake in the United States (US) is 40% higher than that of raw tomato (3025 μg/100 g). Watermelon is the fifth major source of lycopene in the US population's diet *(10)* but in other countries may be equal or even higher than tomato.

Most epidemiological studies report that populations with large intake of fruits and vegetables have lower incidences of degenerative diseases caused by oxidative stress *(5)*.

Canene-Adams et al. *(11)* report that on average, Americans consume about 18 pounds of fresh tomato and about 69 pounds of processed tomato products annually. They also report that according to the Economic Research Services of the United States Department of Agriculture (USDA), canned tomato sauce make up 35% of the raw tomato produced, 18% made into tomato paste, 17% for canned tomatoes, 15% into juice, and 15% into ketchup. Teenage boys consume the highest amounts of ketchup. By increasing age men and women consume more fresh and canned tomatoes and tomato juice. With all the nutrients and phytochemicals and antioxidants contained in tomatoes, this vegetable is a good example of a functional food.

Although tomato and its products are rich sources of β-carotene, folate, potassium, and they are very high in vitamin C, but only their content of lycopene has received attention because tomato and its products are the main source of lycopene in the diet of the US population. Populations of other cultures with a high fruit and vegetable consumption, derive their lycopene and other carotenoids from a variety of fruits and vegetables, and usually have lower incidences of degenerative diseases.

5. ABSORPTION AND UTILIZATION

Lycopene and other carotenoids must be released from the food matrix in order to be absorbed. Carotenoids are less available from raw than from processed fruits and vegetables *(12)*. In tomatoes lycopene is mostly in the layer directly under the skin, thus in cooked pureed tomato and tomato paste it is more bioavailable as a result of the disruption of the plant tissue matrix and dissociation of lycopene-protein complexes, increased surface area, *cis*-isomerization and solubility of lycopene *(13)*.

Karin et al. *(14)* fed mechanically homogenized and heated canned tomatoes to different groups of human subjects. One group received a single dose after fasting and another group were fed equivalent of 22 mg/d of lycopene in processed tomatoes for 4 consecutive days. Carotenoid response in triglyceride-rich lipoprotein fraction of plasma, after single consumption, was compared to that of four daily consumptions by human subjects. They showed that homogenization of tomatoes enhances the lycopene bioavailability significantly. Additional heating of homogenized tomatoes increased bioavailability of lycopene even further. They concluded that other carotenoids had similar responses to homogenization and heating in their bioavailability in humans. Thus it appears that mechanical homogenization and heating of food sources of lycopene and other carotenoids tend to disrupt the plant cells containing the carotenoids

especially lycopene to free these substances making them more bioavailable in gastrointestinal tract of humans.

Mechanism of absorption of lycopene is not well understood. It has been shown that when tomato products are heated and/or pureed by processing, the appearance of lycopene in circulation as well as its concentration is increased specially when heated tomato products are consumed with oil *(15)*. Lycopene, a fat soluble pigment is absorbed and transported in the circulation by the lipoproteins. The geometry of the lycopene has been shown to affect its absorption also. Being hydrophobic, presence of oil with lycopene in foods helps with the absorption of lycopene in the intestinal tract and its transfer in the circulation. Unlu et al. showed that adding avocado or avocado oil to salad or salsa enhanced absorption of lycopene and β-carotene *(16)*. Therefore, it is important to consider adding lipids, preferably unsaturated and specifically ω-3 fatty acid*s*, in preparation and consumption of healthy foods containing lycopene and other carotenoids.

About 90% of the lycopene in foods sources (i.e., fruits and vegetables) is present in *all-trance* configuration. Some *all-trance* lycopene is isomerized to *cis* configuration in the gastrointestinal system, particularly in stomach. Boileau et al. *(17)* in a study feeding a mixture of *cis* and *trans* isomers of lycopene to lymph-cannulated ferrets, reported that the concentration of *cis*-isomers of lycopene was increased 59% in the intestinal mucosal cells and up to 72% in the lymph collections over the *all-trans*-isomers of lycopene, concluding that the shorter length of *cis*-isomers of lycopene increased their solubility in biological systems. They showed that *all-trance* lycopene being a linear, bulky molecule is less soluble in bile acid micelles, not easily absorbed and incorporated into the chylomicrons as does *cis* configuration of lycopene. Some studies suggest that biological and antioxidant effects of *cis*-lycopene may be more than that of *trans*-isomers *(18).* Mayne and colleagues *(19)* derived information about factors that affect the plasma lycopene concentration from participants of two centers that were studying cancer prevention in cross-section population groups. They found that the low plasma concentration of lycopene was related to geographical sites, being unmarried, vitamin C consumption, and gender (women's plasma lycopene was higher than men regardless of intake). They found that the three determining factors of plasma lycopene in the sample population studied were: levels of cholesterol, dietary intake of lycopene, and marital status. Other lifestyle and demographic factors had less importance in determining the plasma lycopene levels. Ganji and Kafai *(20)* analyzed the data from the Third National Health and Nutrition Examination Survey and reported on the determinants of serum lycopene concentration in 3413 subjects. Their analysis showed serum lycopene concentration was not related to race-ethnicity, alcohol consumption, body mass index (BMI), blood pressure, and consumption of non-tomato vegetables, fruits, and juices. On the other hand gender (F < M), age (–), geographical location (South < West), socioeconomic status (–), serum total cholesterol (+) intakes of fat, tomato and tomato products (+), and smoking (–) were significant determinants of serum lycopene.

Erdman *(21)* reviewed the concentration of lycopene and other carotenoids in tissues and indicates that some meats such as liver, adrenals and reproductive tissues may have about 10-fold higher concentration of carotenoids than in other meats. A much higher concentration of more polar carotenoids, lutein, and zeaxanthine, have been found accumulating in

the macular pigment of the retina of the eye. It is speculated that differences in the concentration of lycopene and other carotenoids in the meats may be related to the number of receptors for LDL and their rate of uptake by different meats. Of course, knowing the antioxidant effect of the carotenoids it could be assumed that meat concentration of carotenoids can reflect the relative oxidation or metabolic rate in those meats. Erdman also concludes that lycopene accumulation in some meats is inversely related to androgen status and appears to also be inversely related to energy intake and the absorption and metabolism of carotenoids depends on overall redox state of the meats and "affected by hormonal status of the host."

Edwards et al. studied the bioavailability of lycopene from fresh frozen watermelon juice in a 19-wk crossover study with healthy, nonsmoking adult individuals. The subjects' diet was controlled to be weight maintenance regimen. The study included a period of 2 to 4 wk (wash out) preceding the treatment period. The diet of subjects was restricted in lycopene rich foods. All subjects received either 20.1 mg/d lycopene and 2.5 mg/d β-carotene form watermelon juice (W-20), or control diet with no juice (C-0) interspaced by washout periods. Subsequently, for the third treatment subjects were divided in to two groups. Twelve subjects consumed 40.2 mg/d lycopene and 5 mg/d β-carotene from watermelon (W-40) and 10 subjects received 18.4 mg/d lycopene and 0.6 mg/d β-carotene form tomato juice (T-20). Plasma lycopene concentration showed lycopene to be bioavailable from both tomato juice and fresh frozen watermelon juice with no dose-response effect. Plasma β-carotene concentration was significantly greater after both doses of watermelon juice than tomato juice. The researchers concluded that for lycopene to be bioavailable, no heat treatment is needed for watermelon juice, as it is readily absorbed form fresh frozen juice *(22)*.

6. SUPPLEMENTING FORMULATIONS

Richelle et al. *(23)* in a study with human subject compared a formulation of lactate entrapped lycopene in whey protein called lactolycopene, with a positive control consisting of tomato paste which provided 28 mg/d of lycopene and a placebo diet containing whey protein (negative control). The lycopene from both sources and the whey protein placebo were added to their self selected free diet. They observed that the serum lycopene concentration in both lycopene sources that reached maximum in two weeks and kept constantly up to the eighth week of the study were similar and higher than the placebo group. Lycopene in the serum of both supplemented groups were 40% *trans* and 60% *cis* lycopene although all the lycopene in both supplements consumed were in *all-trance* form, indicating that *all-trance* lycopene was converted to *cis* form of lycopene in the GI tract and was absorbed better. Some of *all-trans* lycopene consumed was isomerized to *cis* upon absorption. They also observed that the meat content of lycopene in buccal mucosal cells followed similar time-concentration curves of lycopene incorporation as in the serum lycopene.

Trumbo *(24)* showed the results of safety tests performed by vitamin manufacturers on the safety of lycopene supplements and believes that although excessive amount of β-carotene consumed can have a pro-oxidant affect; lycopene does not show that effect and is relatively safe in high levels of consumption.

7. BIOLOGICAL FUNCTIONS OF LYCOPENE

Carotenoids including lycopene and β-carotene are transported in human serum by lipoproteins, especially LDL *(25–27)*. This may indicate that their antioxidant effects protect lipoproteins especially LDL from being oxidized by singlet oxygen thus is responsible for lower incidences of myocardial infarction (MI) in population with higher fruits and vegetable intake as reported by Argarwal et al. These investigators found significantly lower serum lipid peroxidation and LDL oxidation in the serum of subjects whose diets were supplemented with tomato juice and other tomato products *(28)*. Oxidative stress in serum lipoproteins, cell membranes of tissues and body organs are known to be the causes of many non-infectious diseases that dietary antioxidants can prevent. Lycopene is one antioxidant that has been studied extensively in prevention of cancers of prostate, lung, and other organs. Hadley's group in feeding human subjects different tomato products showed that the plasma levels of all isomers of lycopene will change rapidly with change in dietary patterns of intake. They also showed that by day 15, consumption of tomato products human subjects showed a significant increased protection of lipoprotein to ex vivo oxidative stress *(29)*. The role of lycopene is discussed in the following section in relation to specific diseases.

7.1. Lycopene and Diabetes

Type 2 diabetes is a disease that is generally caused by the body cells becoming insulin resistance. It is speculated that this resistance of cells to insulin is related to obesity and accumulation of fat in the tissues. Oxidative stress is thought to be associated with the pathogenesis of type 2 diabetes mellitus. This type of diabetes is brought about by causing impairment of insulin secretion form pancreas and resistance of cell to the action of insulin, which may be caused by oxidative stress. Wang et al. studied 35,783 women in the US in a prospective study. They examined the data on intake of lycopene containing foods and association between type 2 diabetes by administering a 131 item validated semi-quantitative food frequency questionnaire at the base line and after 10.2 yr into the study. Results showed that women who consumed greater amount of lycopene in their diet had healthier lifestyle than those who consumed less. They carefully adjusted for all the confounding factors and explained the limitation of their study but concluded that their data did not find significant relation between consumption of lycopene containing foods and type 2 diabetes mellitus *(30)*.

The epidemiological studies investigating the relationship of dietary lycopene and diabetes are scarce. There are however indirect evidence that lycopene can prevent the onset of type 2 diabetes by its antioxidant activity. But direct evidences resulting from research studies are rare. A similar prospective study investigating the relationship between intake of lycopene and risk of type 2 diabetes in a cohort of men and women also found no significant association. This study was conducted in Finland *(31)*. More carefully designed studies are needed to determine if a relationship exists.

7.2. Lycopene in Smokers

Smoking is shown to increase free radicals in the meats of smokers causing oxidation of LDL, leading to foam cell production and atherosclerosis. In some studies the

concentration of lycopene is reported to be lower by 18 to 44% in the plasma of smokers as compared with nonsmokers *(32–34)*. Study of Rao and Argarwal showed that in human subjects postprandial concentration of serum lycopene was lower than serum concentrations in fasting. In the same study, a 40% decrease in serum lycopene concentrations was observed immediately after smoking 3 cigarettes in 30 min. It is believed that lycopene is used to counteract the free radicals produced by meal and smoke induced metabolic stress *(35)*.

Studies were reviewed by Arab and Steck *(36)*; show that cigarette smoking had no effect on the levels of plasma lycopene concentrations. Plasma concentration of α-carotene, β-carotene, lutein, zeaxanthin, cryptozanthin and *cis*-β-carotene, were lower in smokers than nonsmokers at the same levels of intake but the concentration of lycopene in the same subjects did not differ. These investigators found this contrary to expectations and they speculated that lycopene protective mechanism may not result solely from its antioxidant properties. Study by Brady et al. *(37)* demonstrated that although diet of smokers was lower than non-smoker in lycopene, the serum lycopene concentrations were not significantly different between the two groups. Since studies by different investigators have shown mixed results on the effect of smoking on the concentration of lycopene in circulation of human subjects, the relations between lycopene and smoking is not clearly defined.

The contradictory results reported above are believed to result from unrecognized mechanism of action of lycopene and other carotenoids in regards to damages that exposure to smoke can cause in the lungs of the smokers.

Wang *(38)* indicated that in order to better understand the chemoprotective role of lycopene in cancer, the mechanism of action of lycopene must be carefully studied. He has demonstrated that ferrets in studies with lycopene have shown close similarities with humans in terms of absorption and meat distribution of lycopene, firmly believing that ferrets are good models to study and elucidate the mechanism of action of lycopene in protecting humans exposed to tobacco smoke from lung cancer. Wang and colleagues recommend that ferrets be used as an experimental model for study of the protective effects of lycopene against damages caused by cigarette smoke in humans.

He demonstrated that when ferrets' diet was supplemented with lycopene, the concentration of lycopene increased in both plasma and lung tissue significantly. However, the concentration of lycopene was much higher in the plasma. When ferrets were exposed to tobacco smoke, the concentration of lycopene was decreased in both plasma and lung tissue, indicating a strong utilization of lycopene in both resulting from smoke exposure. He also points out the above observation is consistent with the data obtained from the National Health and Nutrition Examination Survey III which also showed lower serum lycopene concentration in smokers than nonsmokers *(39)*. Recently it has been reported that "the Apo-10'-lycopenoids mediate the chemoprotective activity of lycopene by inhibiting cell proliferation and modulating the action of extracellular signal–regulated protein kinase in A549 lung cancer cells *(40)*."

Liu et al. *(41)* supplemented the diet of ferrets with two levels (high dose vs low dose) of lycopene both with 9 wk exposure to cigarette smoke and without. Ferrets in low-dose lycopene group received 1.1 mg/(kg/d) of lycopene equivalent of 15 mg/d in humans, slightly higher than mean lycopene intake in the US population. The diet of ferrets in high dose group was supplemented with 4.3 mg/(kg/d) of lycopene which is

equivalent of 60 mg/d in humans. The results showed increases of lycopene concentration substantially in both plasma and lung tissue by both low-dose and high-dose supplementation over the 9 wk. These increases mirrored that in humans under similar supplementations. Exposure of ferrets to cigarette smoke decreased the concentration of lycopene in plasma of both high does and low dose ferrets about 40% and the decrease of lycopene concentration in lung tissue of both groups was about 90%.

These studies make it possible to assume that lycopene and other carotenoids have a positive protective role against oxidative damage to lungs and other meats caused by cigarette smoke. That is not to be interpreted that supplementing the diet of smokers with lycopene, carotenoids, and other antioxidants can protect individuals against oxidative damage and the risk of cancer brought about by smoking. The best protection against oxidative damage and consequences there of is not being exposed to the damaging substances, in other words preventing the causes of damage is the best protective way.

7.3. Lycopene and Cardiovascular Diseases

There are numerous studies that show the relationship between consumption of fruits and vegetables and coronary heart disease (CHD). Intake of fruits and vegetables may help in reducing CVD by the presence of many protective factors like antioxidants, nonantioxidant phytochemicals, fiber, potassium, magnesium, vitamins, and other still to be discovered substances in fruits, specially brilliantly colored ones such as berries, tomatoes, melons, and citrus fruits. It is generally agreed that consumption of whole foods will be more beneficial that the individual nutrients. Therefore, the focus of this section is to concentrate on reports of the studies on intake of these foods and role of the fruits and vegetables providing carotenoids especially lycopene in protection against cardiovascular disease (CVD).

Lycopene was shown to act as a hypocholestrolemic agent in the J774A.1 macrophage cell line by inhibiting HMG-CoA reductase pathway *(42)*.

Rao and Agarwal *(43)* have reviewed the antioxidant role of lycopene in heart disease and cancer. They observe that lycopene is a potent antioxidant that prevents atherogenesis and carcinogenesis by protecting critical molecules such as lipids, low density lipoproteins, proteins, and DNA. In in vitro studies it has been shown that lycopene inactivates hydrogen peroxide, scavenges and deactivates nitrogen dioxide, thiyl and sulphonyl radicals. By being highly lipophilic lycopene is usually found within cell membranes and other lipid components of the cell. Therefore, it is expected to have maximum ROS scavenging effect in the lipid environment of the cells. It has been shown to have a very high antioxidant effect in protecting 2, 2′-azo-bis (2, 4-dimethylvaleronitile) induced lipid peroxidation of the liposomal membrane. Lycopene and other carotenoids can inhibit LDL oxidation in vitro. Lycopene also protects lymphocytes against NO_2^- induced membrane damage and cell death twice as effectively as β-carotene.

Ressanen et al. *(44)* showed that when adjusted for energy, intake of nutrients: fiber, folate, vitamins C and E, β-carotene, lycopene "attenuated the protective effect of intake of fruits, berries and vegetables against mortality; thus it may be the main protective nutrients in these foods." Accordingly they indicate that energy-adjusted intake of vitamins C and E, folate and lycopene from fruits, vegetables, and berries can explain 28% of protection against all-cause mortality, and intake of folate, vitamins C and E explains

36% protective effect of cardiovascular mortality. They also reported that in these groups of subjects maximum oxygen intake during the exercise test had a strong correlation with intakes of fruits, berries, and vegetables. They pointed out that those men consuming higher amounts of plant products had a healthier lifestyle and included exercise in their daily life. These investigators emphasized the importance of including not only the recognized risk factors when studying the association between nutrients and diseases, but considering all factors that effect lifestyle including exercise.

The preventing role that lycopene may play in CVD can result from the fact that hydrocarbon carotenoids, including β-carotene and lycopene, are transported in LDL. Therefore, because of the position of these carotenoids in the LDL molecule, they can protect LDL from oxidation *(45)*. Studies have also shown that adipose tissue concentration of lycopene is associated with protection against myocardial infarction (MI). The highest concentration in the adipose tissue is most protective in nonsmoking men.

Although the biological activity of lycopene is complex and requires extensive studies to be explained, there are some interesting studies reported in the literature worth mentioning in this review. Fuhrman et al. *(42)* reported of the non-antioxidant function of lycopene both in vitro and in humans. They added lycopene to macrophage cell lines in vitro and showed a decrease of 73% in cholesterol synthesis and an increase in LDL receptors. Incubation of cells with lycopene caused increased degradation of cell LDL of 34% and about 110% raise in LDL removal from the circulation. Same investigators in a human study with 6 men showed 14% reduction in plasma LDL when feeding 60 mg/d of lycopene, equal to the amount of lycopene in one kilogram of tomato/d; without any significant change in plasma HDL concentration in the subjects of this study. These investigators suggest about a 30 to 40% reduction in risk of myocardial infarction as a result of consuming as high an amount of lycopene regularly, as was tested above.

Romanchik et al. *(46)* enriched LDL samples isolated from serum of 5 subjects, with β-carotene, lycopene, and lutein. They found that when copper mediated oxidation of LDL was used, before substantial amounts of lipid peroxidation products were formed, carotenoids were destroyed in the samples. This experiment further provided evidence of antioxidant functions of these carotenoids.

In an abstract Street et al. *(47)* reported some trends in the serum concentration of lycopene in subjects, measured 7 to 14 yr before unset of myocardial infarction (MI). But no does-response and no significance was found in the above relationship, in spite of the fact that in the lowest quintile of serum lycopene concentration there appeared to be a risk for MI. The authors found that the inverse relation between serum lycopene and risk of MI was seen more in the smokers than nonsmokers.

Sesso et al. found no association between plasma lycopene concentrations and CVD in a nested case-control study of the subjects from physician's Health Study. These investigators found their results of the study inconsistent with those of other reports of case control studies showing an inverse relation of lycopene with CVD. They attributed this difference to the fact that the study subjects were at least 10 yr older than those in other studies and the plasma lycopene levels may not reach high enough in the elderly to make a difference *(48)*.

Rissanen et al. *(49)* in the Kuopio Ischemic Heart Disease Risk Factor study reported that the serum lycopene was inversely related to incidences of acute coronary events and stroke. They also observed that as the serum concentration of lycopene increased by

0.01 μmol/L, the risk of acute coronary incidences and stroke was lowered by approx 4%. They categorized their subjects according to the serum levels of lycopene as well as age, systolic pressure, BMI, serum Folate, vitamin C, and β-carotene. They compared the rate of development of acute coronary events and stroke in subjects in the lowest serum lycopene quartile with those in other quartiles at midpoint of the study (after 63 mo). Results showed that 9.7% of men in the lowest serum lycopene concentration quartile developed acute coronary events vs only 2.8% of subjects in other three quartiles ($p < 0.001$), and 2.7% of the subjects in the lowest quartile developed stroke vs 0.6% in the other quartiles combined ($p < 0.016$). In using Cox' proportional hazard's model adjusting for the above factors men in the lowest quartile of serum lycopene concentration had a 3.3-fold higher risk of acute coronary events and stroke compared with the rest of the subjects.

Concentrations of lycopene in adipose tissue also has an inverse relationship to the to the risk of MI. Gomez-Aracena et al. *(50)* reported the risk of MI for the highest quintile of the adipose lycopene concentration was 60% lower than the lowest quintile. This was after data was adjusted for age, family history of CHD, and cigarette smoking. Results of data from the total multinational, multi-center EURAMIC confirmed this finding. The risk of MI was 48% reduced in men who had the highest concentrations of lycopene in their adipose tissue biopsies as compared with the men with the lowest levels of lycopene in their adipose tissues *(51)*. Results of these studies emphasize the importance of the dietary carotenoids especially lycopene in protecting against CVD both in men and women. Although there needs to be more conclusive studies to be able to state with certainty that these compounds in the diet can prevent CVD, however, it is easier but much more expensive to prevent these diseases by medications such as cholesterol lowering drugs (statins). Quoting a physician here: "drinking water should be enriched with statin drugs since almost everyone needs them" shows neglect of the importance of dietary intervention in health promotion by medical professionals. It must be emphasized to the medical and other health professionals that nutrition education, especially including and increasing consumption of fruits and vegetables in everyday meals and snacks provide the body with phytochemicals and antioxidants contained in fruits and vegetables, can do a better job of preventing degenerative disease than medication, it is much less expensive and causes no side effects.

7.4. Lycopene and Cancer

Free radicals, ROS and other oxidants, present in the meats as by product of normal metabolism, can produce promutagenic lesions in DNA that if not repaired can cause cancer. Components of fruits and vegetables high intake of which is associated with lower incidence of cancer including vitamins C, E, the carotenoids, and polyphenolic compounds in plants may increase cellular defense and protect cellular components from oxidative damage. It is suggested that some of these protective dietary compounds have biological activities not related to their antioxidant effect that make them able to modify gene expression thus repair damaged DNA preventing mutation and cancer. Lycopene is one of these components of fruits and vegetables that may be able to accomplish this by enhancing gene expression and DNA repair prior to DNA replication. Whether is lycopene able to modulate DNA repair in addition to its antioxidant function is an active field of research at present *(52)*.

Lycopene with its anti-oxidant ability has been shown to reduce the risk of different types of cancer by preventing oxidative changes in DNA and RNA. Lycopene has also been credited with prevention of cell proliferation, migration and invasion in cancer. Pastori's group showed that lycopene and α-tocopherol at physiological concentrations inhibited, synergistically, cell proliferation of an androgen insensitive prostate carcinoma cell line *(53)*.

The protective role of lycopene in prevention of chronic diseases and interest it created in the research community is derived from data obtained in both epidemiological and clinical studies that estimated daily lycopene intake of communities or measurements of plasma lycopene in clinical studies in relation with chronic diseases such as cancer and CVD. Golditz et al. *(54)* in a prospective study investigated the frequency of intakes of vegetables of different kinds and cancer deaths in 1271 elderly residences of Massachusetts and showed that high intake tomato decreased mortality from cancer by 50%. In this study intakes of carrots and other carotenoid rich vegetables had no beneficial effect.

By being a strong antioxidant, lycopene is shown in the studies to protect against many types of cancer. Lycopene has been shown to be a stronger inhibitor of the growth of human endometrial, mammary, and lung cancer cells grown in cultures, than α- or β-carotene, "lycopene and vitamin D_3 together synergistically inhibit cell cycle progression and induced differentiation of the HL60 promyelocytic leukemia cell lines *(55)*." Mouse embryo's fibroblasts enriched with lycopene up-regulated gap-junction-communication by increasing the expression of the connetxin 43 gene. This gene is known to encode a major gap-junction protein, acting as an anti carcinogen. Lycopene protects against microcystein CR-induced mouse hepatocarcinoma, arresting cells in C0/C1 phase of the cell-cycle by suppressing the phosphorelation of regulatory protein. Lycopene also is shown to reduce cellular proliferation induced by IGF-1 in cancer cell lines.

Huang et al. *(56)* studied the effect of lycopene on cell migration and invasion (metastasis), the most important cause of cancer deaths, and investigated the mechanism of its action by using a highly invasive hepatocarcinoma cell line. They showed that lycopene inhibited the cancer metastasis by up-regulating the expression of nm23-H1, a metastasis suppressor gene in a hepatoma cell line, SK-Hep-1. In their study, migration and invasion was inhibited by lycopene in a bell-shaped fashion, 5 μmol/L of lycopene causing 91 and 63% inhibition of migration and invasion respectively. They also showed that lycopene is almost 8 times more effective than β-carotene in reducing cell invasion. They observed that lycopene enhanced nm23-H1 expression at both protein and mRNA levels, concluding that lycopene has "a significant anti-migration, and anti-invasion activity and that this is associated with its induction of nm23-H1expression."

7.5. Lycopene and Prostate Cancer

Nelson *(57)* blames animal fat, charred or well-done red meats that may contain "prostatic carcinogens" for increased prostate cancer risk. He reviews studies in which feeding male rats heterocyclic aromatic amine 2-amino-L-methyl-6-phenylimidazo [4,5- L]pyridine, a carcinogen formed in overcooked meats caused mutation in the DNA of the prostate cells leading to prostate cancer in those rats. He also mentions chronic prostatitis, sexually transmitted infections, or even inflammatory response to infections can be the cause of prostate cancer when the cellular defense mechanism is inadequate against the reactive

oxygen and nitrogen species. Research has indicated that intake of antioxidant and carcinogen detoxifying foods containing "detoxification enzyme inducer" foods such as vegetables high in sulforaphane, use of anti-inflammatory drugs and treatment of any infection before it becomes chronic could be a good strategy in prevention of prostate cancer. Campbell et al. *(58)* in an interesting discussion and review of epidemiological, clinical, and laboratory studies present data from their own and other laboratories that support the role of not only lycopene in tomato but other antioxidants, phytochemicals, Polyphenols, minerals, and vitamins in tomato that can be responsible for reducing risk of prostate cancer. They studied "the antiproliferative effect of tomato polyphenols in a human prostate cancer cell line, LNCaP, and in mouse hepatocyte cell line Hepa1c1c7 and showed that "after treatment for 48 to 72 h, tomato aglycone polyphenols, including quercetin, kaempferol, and naringenin, inhibited cancer cell proliferation in both LNCaP and Hepa1c1c7 cells in a dose–dependent manner (10–50 μmol/L) without having cytotoxic effect." But the glycone polyphenols, rutin, quercetrin and narengin did not decrease cell growth at much higher concentrations. They suggested that LNCaP and Hepa1c1c7 may not respond to glycosylated forms of the polyphenols. These investigators also indicate in addition to lycopene, other nutrients in tomato may also have a significant role to play in reducing or preventing the risk of prostate cancer. Tomato antioxidants, phytochemicals, and polyphenols also been shown to inhibit cytochrome P450-1A isoforms and others have shown "tomato phytochemicals modulate hormones and growth factor signaling in prostate cells."

The effectiveness of lycopene in preventing prostate cancer has been the subject of numerous studies and has put tomato, pizza, and other tomato products, and lycopene containing foods on the map as prostate cancer preventing foods for past few years. It is believed that cancer preventive effect of lycopene is attributed to its strong antioxidant effect in protecting oxidation of lipids, lipoproteins, proteins and DNA. It is logical to hypothesize that lycopene with the abundance of conjugated unsaturated sites in the molecule can by quenching the free radicals inhibit the oxidation and damage to DNA in the prostate cells thus preventing the risk of prostate cancer.

Many epidemiological, clinical and case control studies have been reported in the last few years and results are mixed at best and not conclusive. Kristal and Schenk *(59)* reviewed the published literature and observe that only one prospective cohort, the Health Professional Cohort study *(60)* conducted by Geovannecci et al. found that consumption of cooked tomato products reduced the risk of prostate cancer a significant 23% and proportional decreased risk with higher consumption of tomato products. These investigators also found a 16% risk reduction with respect to the consumption of high dietary lycopene in the same cohort subjects.

Study of intake of tomato and its product with beans lentils and peas by 14,000 Seventh Day Adventist men showed a decreased risk of prostate cancer in the group *(61)*. In the Netherlands *(62)* cohort study by Shuurman et al. reported no association between consumption of lycopene and risk of prostate cancer. In the Physician's Health Study the investigators found that elevated serum lycopene was associated with 41% reduction in risk of prostate cancer in the placebo group *(63)*.

Kristal and Schenk *(59)* report of six other cohort studies that found no statistically significant association between serum lycopene concentration and risk of prostate cancer. They continue to examine other studies that have produced mixed results resulting from

either not having the right design, or not enough participants or the methods of assessment of serum lycopene needed to be improved to provide meaningful conclusions as to the association between the effect of lycopene and reduction in the risk of prostate cancer. They also point out the problems inherent with lycopene exposure such as variability of food content of lycopene, absorption, and bioavailability of lycopene making serum levels or intake of lycopene the factors measured in the studies that are not the best marker of prostate tissue concentration of lycopene which is really what should be investigated but it is not a feasible method for the epidemiological studies.

Other investigators have reported of studies that have produced positive results showing an inverse association between intake of lycopene and risk of prostate cancer.

The US Health Professionals follow up study in which intake of carotenoids and retinol from a food frequency questionnaire was evaluated showed an inverse relationship with intake of lycopene from tomato products and the risk of prostate cancer *(64)*.

Lu et al. *(65)* in a case-control study investigated the effects of plasma carotenoids including lycopene, retinol, α-and γ-tocopherols on the risk of prostate cancer. They enrolled 65 patients with prostate cancer and 132 healthy cancer free men as controls. The study lasted 4 yr. The researcher adjusted the data for age, race, years of education, daily caloric intake, smoking, use of alcohol and family history of prostate cancer, found significant inverse relation between incidences of prostate cancer and plasma concentration of carotenoids: lycopene, zeaxanthin and border line associations were found for lutein and β-cryptoxanthin and no association were found for α- and β-carotenes, α- and γ-tocopherols and retinol when comparing highest and lowest quartiles. They reported 83% prostate cancer risk reduction in those subjects with highest plasma lycopene concentration compared with those with the lowest plasma concentration of lycopene. This association was found to be dose-responsive. They also reported an inverse association of plasma lutein, zeaxanthin and β-cryptoxanthin with prostate cancer.

One of the major problems in men over 50 years old is a condition called benign prostate hyperplasia (BPH). It is not known if tomato products or lycopene has any effect on the growth of the normal prostate cells that causes BPH. It is believed that diet may play a role because, similar to CVD, BPH also is a Western disease and relatively rare in the Eastern especially Asian countries. Epidemiological studies of Asian men who migrated to the US and adopted Western patterns of eating showed an increased risk of BPH, prostatitis, which are both precursors of prostate cancer and prostate cancer *(66)*. Because lycopene is the major carotenoid in the human prostate gland in amount higher than other meats, and has some beneficial effect on reducing the risk of prostate cancer one can assume that it may have some preventive role in BHP and prostatitis as well. Obermuller et al. *(67)* treated normal human prostate epithelial cells with synthetic *all-trance* lycopene and showed that lycopene inhibited the growth of human prostate epithelial cells significantly, in a dose dependent response. A significant cell-cycle arrest was in the G0/G1 phase was observed as a result of lycopene inhibition of cycle D1-protein expression but cycle E levels did not change. They hypothesized that since lycopene inhibits growth of non-neoplastic prostate epithelial cells in vitro, it may as well do the same in vivo.

Siler and coworkers *(68)* using MatLyLu dunning prostate cancer model fed rats diet supplemented with lycopene and vitamin E and showed that lycopene and vitamin E were accumulated in the tumor tissue and the necrotic areas in tumors of the rats were

increased significantly after 18 and 14 d of feeding respectively. They also showed that lycopene and vitamin E both suppressed genes responsible for steroid metabolism and signaling. Lycopene feeding caused reduction of steroid 5-α-reductase 1 expression. This enzyme converts androstenedione and testosterone into 5 α-dihydrotestosterone. In addition to 5-α-reductase down-regulation lycopene also down-regulated a series of androgen target genes. These investigators also showed that in addition to the above effect lycopene and vitamin E in combination and alone suppressed other factors related to inflammation or immune response in prostate cells both in tumor tissue and in healthy prostate tissues.

Clinton *(69)* believes that as a result of the interest raised in consumption of tomato product in respect to its benefits in reducing prostate cancer risk, it is important to keep in perspective that more statistically powerful studies are needed to provide concrete evidence of the effectiveness of tomato products, and lycopene specifically, in prevention of prostate cancer. In the meantime, advocating increased consumption of tomato and its products must fall within the recommendations put forward by the Nutritionists and other Health Professionals to increase daily intake of all fruits and vegetables as a healthy lifestyle practice for all people.

7.6. Lycopene and Other Cancers

7.6.1. Lycopene and Colorectal Cancers

The reason for development of colorectal adenomas, precancerous polyps has been subject of much debate among scientists. Dietary fiber, greater consumption of fruits and vegetables, and reduction of fat in the diet have shown both positive and negative relations to colorectal polyps or cancer in studies reported in the past few decades. This fact has caused the suggestion that other dietary nutrients and components of high fiber foods (e.g., fruits and vegetables) may affect the incidences of colorectal adenomas or cancer *(70)*. Case–control studies in Italy has consistently shown that high consumption of tomato and its products reduce the risk of colorectal cancer as well as entire gastrointestinal tract *(71)*. Erhardt et al. *(72)* studied the association of plasma lycopene, β-carotene and α-tocopherol concentrations and colorectal adenomas in a case control study. They included 73 patients with adenomas and 63 individuals without any polyps and 29 patients with hyperplastic polyps in the study. Dietary histories, smoking, and alcohol consumption of the subjects were colleted. They found that in patients with adenomas median plasma lycopene concentrations were significantly lower than in control group ($p = 0.01$). The plasma median β-carotene concentration was also lower but not significantly in the adenoma patients. Patients with hyperplastic polyps were similar with the controls in all variables. They concluded that lycopene provided protection against adenomas in subjects with a high intake of tomatoes and tomato products. These investigators only related the plasma lycopene and β-carotene to the presence of adenomas. Nair et al. *(73)* measured the seven dietary antioxidants including lycopene in the mucosal biopsy samples of ten patients with adenomatous polyps and fifteen control subjects without polyps. They showed that meat concentrations of antioxidants were generally lower in patients with adenomatous polyps than that of controls. From these two small studies it can be concluded that although not enough studies have proven, antioxidants, and lycopene can be protective against colorectal cancer.

7.6.2. Lycopene and Pancreatic Cancer

Some epidemiological studies have suggested that inclusion of high amount of fruits and vegetables in the diet may reduce the risk of pancreatic cancer. Pancreatic cancer is one of the types of cancer with a very low survival rate, less than 1% in 1 yr after the diagnosis and the five year survival rate being less than 5% *(74,75)*. Experimental investigations have provided substantial evidence that the carotenoids in fruits and vegetables play many roles in preventing cancer by their antioxidation properties, enhancing immune function of the body, stimulating gap junction and intercellular communication, inhibition of cellular proliferation and induction of detoxifying enzymes *(76,77)*.

Nkondjock et al. *(78)* studied the carotenoid intakes of pancreatic cancer patients from the cancer registries in 8 of 10 provinces of Canada. In this study food consumption data were obtained using a semiquantitative food frequency, and Willett questionnaire *(79,80)*.

Data from 475 cases (264 men and 211 women) and 5039 controls (2547 men and 2492 women) were collected and analyzed. These analyses showed that among the cases use of tobacco was very high ($p < 0.01$). Data showed that 2 yr before the diagnosis of cancer in both men and women with higher BMI, the risk of pancreatic cancer was higher. In men the cases had more energy intake than the controls. Investigators found a significant inverse relationship between lycopene intake and pancreatic cancer in men when they compared the highest quartile of intake with the lowest. Intake of other carotenoids was not associated with the risk of pancreatic cancer. They also observed a significant inverse relationship between the risk of pancreatic cancer and intake of β-carotene and total carotenoids in those patients who never smoked tobacco.

As it is evident form the role of lycopene and carotenoids in reduction of risk of cancers other than prostate cancer, little research data is available and more work needed to elucidate this role for both lycopene and other carotenoids.

8. CONCLUSIONS

From the above literature, it is evident, that fruits and vegetables have varieties of beneficial effect in the health promotions and disease prevention among humans by the virtue of the functional substances that they provide to the body. Wealth of antioxidants, phytochemicals, vitamins, minerals, and fiber, and maybe still unidentified substances in these foods justifies calling them functional foods. This chapter focused on carotenoids and lycopene specifically in relation to cancer and CVD, elucidating the preventive and risk reduction effects of these compounds. It is evident that lycopene in fruits consumed in adequate quantities is effective in reducing risk of some cancers, especially prostate cancer and prostate hyperplasia. The mechanism of action of lycopene is not completely described but it is a very active area of research. Future literature will surely bring about more information on this subject. The role of lycopene and other carotenoids in reducing risk of CVD seem to be in its antioxidant effect and preventing the oxidation of lipoproteins, inhibiting the enzymes involved in cholesterol synthesis, and increasing LDL receptors in meats so that LDL is cleared from circulation faster.

There are still functions and mechanism of action of carotenoids to be found, particularly lycopene in preventing diseases and promoting health. In the light of what are proven facts about the beneficial effect of these components of fruits and vegetables it

is important to encourage increased consumption of these foods in every day diet as one of many steps needed for attaining a healthy lifestyle.

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31 Dietary Intake of Lycopene and Risk of Prostate Cancer

Paula Inserra

Abstract

Recent evidence suggests a role for the plant compound lycopene in the prevention of prostate cancer. Lycopene is a carotenoid that lacks provitamin A activity and acts as a potent antioxidant in the human body. In the United States, the richest sources of lycopene are tomato products. Processed tomato products are a particularly rich source due to the higher bioavailability of lycopene in its *trans* configuration. Prostate cancer is the second leading cause of cancer death in the United States for men and is significantly higher among African Americans. Since lycopene has been shown to concentrate in the prostate gland, several investigations have been conducted in an attempt to ascertain lycopene's role in the prevention of prostate cancer. This chapter will therefore review the recent evidence for lycopene and prostate cancer prevention, particularly focusing on the methods of assessing lycopene status.

Key Words: Lycopene; tomatoes; tomato products; cancer; prostate; diet.

1. INTRODUCTION

Recent evidence supports a role for the plant compound lycopene in the prevention of prostate cancer. Lycopene is the predominant pigment in strawberries, watermelon, and tomato products that imparts the rich red color to these foods. Lycopene is a carotenoid, related to β-carotene, that lacks provitamin A activity. In plants, lycopene exists mainly in its *trans* configuration which is its more stable form *(1)*. In humans, however, lycopene is found in both the *cis* and *trans* configurations. In fact, the *cis* isoform is more bioavailable *(1)*. In the United States (US), food sources that contribute the highest amounts of lycopene to the American diet include, ketchup, tomato juice, and pizza sauce *(2)*. Furthermore, Americans eat almost four times more processed tomato products than they do fresh tomatoes *(2)*. Unlike most food processing that leads to nutrient loss, processing of tomato products results in enhanced bioavailability of lycopene. During processing, lycopene is converted from its *trans* isoform to its *cis* isoform. Encouraging increased consumption of these widely accepted American foods would be well received and could potentially lower the risk of men developing prostate cancer and impart other health benefits to the general population.

Lycopene is a potent antioxidant. It is particularly effective at quenching singlet oxygen, which is generated as a consequence of normal metabolism. Its ability to interact

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with singlet oxygen, is two times as effective as β-carotene and up to ten times as effective as α-tocopherol *(3)*. Plasma levels of lycopene are not necessarily reflective of dietary lycopene intake; however it is the major serum carotenoid *(4)*. Lycopene is found at higher levels in certain human tissues and glands, including the prostate *(5)*. In these tissues it thought that lycopene functions in a variety of ways to impart its protective effects on cancer cell development. The most obvious being its role as an antioxidant. Increased levels of reactive oxygen species (ROS) without effective mechanisms and compounds to deal with them is recognized as a major contributor to the development of cancer. As one of the most potent antioxidants, along with the fact that it is known to concentrate in tissues likes the prostate, leads many investigators to postulate that lycopene consumption may decrease the risk for prostate cancer development. In addition to its role as an antioxidant, lycopene may have other mechanisms of action that impede prostate cancer development *(6)*. In a recent review by Herber and Lu *(7)* lycopene has also been shown to have several other potential means as an antiprostate cancer agent. For one, it was shown to impede growth factor function. By interfering with growth factor function, lycopene may function to inhibit prostate cancer cell proliferation *(7)*. This could potentially slow the progression of prostate cancer development, as well as be a useful adjuvant in the treatment of the disease. In this review the authors also discuss another potential mechanism that may contribute to lycopene's protective role in prostate cancer development. Lycopene has been shown to up-regulate connexin 43 gene expression *(7)*. Connexin 43 is a protein that functions in intercellular communication. Connexin 43 has been shown to be impaired or suppressed in several cancer types.

2. REVIEW OF THE LITERATURE

Because tomato products are the richest dietary sources of lycopene several investigations have looked at the effect of tomato and tomato product consumption has on prostate cancer development. Other investigations have studied the effect of lycopene supplementation and biomarkers of lycopene intake have on prostate cancer development. In studies that report inverse associations between tomato and tomato product consumption and prostate cancer risk, it is important to recognize that although lycopene's role as an antioxidant is well established, other components in tomato products, for instance, vitamin C, folate and other phytochemicals, like β-carotene may also contribute to the lower risk of prostate cancer observed among frequent consumers of tomato products. Additionally, these compounds may work in combination to produce additive, or even synergistic effects, which would then render lycopene supplementation ineffective at protecting against prostate cancer. This difference may be a factor in why some investigation failed to find a protective effect of lycopene and prostate cancer. Upon review of the recent literature on lycopene and prostate cancer prevention, this chapter focuses specifically on the method of assessing dietary lycopene intake and/or biomarkers of lycopene status. The following is a review of the recent finding regarding lycopene and prostate cancer risk.

In 1995, Giovannucci et al. evaluated the relationship between dietary carotenoids and prostate cancer risk *(8)*. The authors found that despite studying the effect of 46 different fruits and vegetables, tomatoes, tomato sauce, pizza, and strawberries were the only ones significantly associated with lower risk for developing prostate cancer *(8)*.

Further analysis also demonstrated a protective effect for lycopene intake itself, whereas no association was seen for the other carotenoids *(8)*. In 1999, a follow up to this initial study was conducted *(9)*. In this nested case-control analysis, an inverse association was found for plasma lycopene levels and prostate cancer *(9)*. Once again, the authors report this protective effect for lycopene only, and not for the other carotenoids *(9)*. They added that the inverse association was even stronger for more aggressive prostate cancers *(9)*. In 2002, the authors conducted another case-controlled study, this time utilizing multiple dietary assessments *(10)*. Unlike previous studies, they averaged results from three different dietary questionnaires. Lycopene, as well as tomato sauce, were associated with reduced risk of prostate cancer *(10)*. In their next case-control study a significant inverse association between plasma lycopene levels and prostate cancer risk was observed, but this effect was limited to those individuals 65 yr-of-age or older *(11)*. In these studies conducted by Giovannucci and his colleagues, dietary measures of lycopene intake showed stronger associations with decreased prostate cancer risk *(8–11)*. These results are therefore not surprising, since we know that plasma lycopene levels are poor indicators of lycopene status.

In another case-controlled study, plasma levels of six different carotenoids were measured in cases and controls *(12)*. After adjusting for age, race, education, calorie intake, family history, smoking, and alcohol use, the authors found significant inverse associations between lycopene as well as another carotenoids; zeaxanthin *(12)*. This investigation further substantiates the results mentioned above.

In a report of two nested case-controlled studies, plasma concentrations of carotenoids, including lycopene, as well as levels of tocopherols, vitamin C retinol and reninyl palmitate were investigated *(13)*. This investigation was nested within the Health Professionals Follow-Up Study and included 450 cases and 450 matched controls *(13)*. Unlike the previous case-controlled studies mentioned above, the authors failed to find a protective association between lycopene and prostate cancer, however they did find a strong inverse association between γ-tochopherol and prostate cancer risk *(13)*. This discrepancy could result from utilizing plasma levels of lycopene as a measure of lycopene status.

As mentioned earlier, plasma levels of lycopene are not an accurate biomarker of lycopene intake. The more consistent associations observed when measuring dietary intake of lycopene, may more accurately assess total body lycopene stores. These investigations underscore the importance of utilizing appropriate biomarkers for assessing nutrient status.

In a more recent study, the association between lycopene intake and intake of specific tomato products and prostate cancer risk was conducted *(14)*. This report was part of the multicentered Prostate, Lung, Colorectal, and Ovarian Screening Trial. Dietary intake was assessed by a 130 7-item food frequency questionnaire at baseline *(14)*. Lycopene intake was not found to be linked with prostate cancer risk nor was number of tomato servings. Some tomato based food items (e.g., pizza and spaghetti sauce) were found to have an inverse association with prostate cancer risk, although it was not statistically significant *(14)*. Interestingly, men with a family history of prostate cancer had a decreased risk of prostate cancer as lycopene consumption increased as well as with some tomato based food items, namely, spaghetti, pizza, and lasagna *(14)*. It is postulated that the protective effects of Lycopene might be marginal and would therefore be difficult to ascertain.

In another investigation a meta-analysis was conducted. Eleven case controlled studies and 10 cohort studies or nested case controlled studies were used in the analysis *(15)*.

The purpose of this investigation was to determine if tomatoes and tomato products are related to decreased risk of developing prostate cancer *(15)*. These authors found only modest effects of lycopene and lycopene rich food sources in relation to prostate cancer risk *(15)*. Additional dietary sources of lycopene could have been missed with this approach to measuring lycopene status.

These conflicting results underscore the importance of identifying all the potential sources of lycopene in the diet. Additionally, they indicate that lycopene's protective effects may be small making it even more essential to accurately assess dietary intake as well as choosing appropriate biomarkers.

Prostate cancer is not only the second leading cause of cancer death among men, but it is particularly high in African-Americans (63/100,000) as compared with Whites (25.8/100,000). In a study by Vogt et al., the risk of prostate cancer in both African-American and White men in the US was investigated *(16)*. In this investigation serum levels of carotenoids were measured in 204 cases and 228 controls *(16)*. It was a multicentered study that was conducted that included 127 African-American prostate cancer cases and 147 White prostate cancer cases *(16)*. Lycopene was shown to protect against prostate cancer particularly for those with aggressive disease *(16)*. This pattern was similar for both African-American and White men. Interestingly, serum lycopene concentrations were found to be significantly lower in the African-American male controls as compared with the White male controls *(16)*. The authors suggest that the great disparity seen in prostate cancer mortality may result from differences in lycopene status *(16)*.

In addition to lycopene's antioxidant properties, lycopene could potentially slow the progression of prostate cancer development by inhibiting growth factor function as well as connexin 43 gene expression, the protein that functions in intercellular communication *(7)*. These properties could prove useful in as an adjuvant treatment modality. In view of lycopene's potential mechanism of action outside of it's antioxidant properties, several investigators have examined if lycopene could improve the prognosis of individuals already diagnosed with prostate cancer. In vitro evidence suggests that lycopene could indeed affect the course of the disease. Proliferation of transformed cells was shown to be halted as gap junction communication is restored *(8)*. In human prostate cancer, disease severity is proportional to the degree of connexin 43 expression *(17)*. Furthermore, in cell lines where connexin 43 expression is restored in human prostate cancer cell lines, cells became more differentiated and had reduced growth rates *(17)*. In view of this in vitro evidence a clinical trial, subjects with prostate cancer were supplemented with 15 mg of lycopene twice/d for 3 wk prior to radical prostatectomy *(18)*. These authors found that connexin 43 expression was higher in the lycopene supplemented group, although it did not reach statistical significance.

Kucuk et al. conducted another clinical trial to determine the effects lycopene supplementation would have in patients with localized prostate cancer *(19)*. Patients were supplemented with 30 mg of lycopene for 3 wk prior to radical prostatectomy *(19)*. Prostatectomy samples were evaluated. Subjects receiving lycopene supplementation had smaller tumors and less involvement of surgical margins. Furthermore prostate specific antigen levels (PSA) were also lower in the lycopene supplemented group. Additional studies investigating the effects of lycopene supplementation in the form of tomato products have on PSA levels may be a cost-effective way to further establish lycopene's protective effect.

3. CONCLUSION

Prostate cancer is the most commonly diagnosed cancer and is the second leading cause of cancer mortality in men living in the US. It is a particular public health concern for African-American men, whose risk is significantly higher than in Whites. It is therefore essential to identify risk factors that both protect and contribute to the disease in hopes to improve the health of the population. Lycopene, as well as other carotenoids and antioxidants show promise as protective agents against this disease and warrant further investigation. Careful attention should be given when designing studies, specifically when assessing dietary intake and choosing the most appropriate biomarker for the nutrient under investigation so as to ascertain the most accurate information.

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IV Wild-Type Food in Different Cultures: Health Benefits

32 Local Wild Foods in the Mediterranean Countries

From Micronutrients to Healthy Effects

Francesco Visioli, Franca Marangoni, and Claudio Galli

Abstract

Throughout the generations, the human genome has adapted to the consumption of wild organisms that in turn adapted to the environment as food sources. In the Mediterranean area wild plants are rich in bioactive compounds and both wild plants and animals have favorable fatty acid profiles in terms of nutritional and health promoting properties.

Key Words: Mediterranean diet; wild plants; bioactive compounds; olive oil; oleic acid; α-linolenic acid; fats in wild animals.

1. INTRODUCTION

1.1. Wild Foods in Human Nutrition in the Preagricultural Era

Evolution is the result of ongoing interactions between a species' genome and its environment over the course of multiple generations. Genetic traits may be positively or negatively selected whether there is concordance or discordance with environmental pressures relative to selection. In particular, when the environment remains relatively constant, genetic traits that are optimal for the population tend to be maintained by stabilizing selection. In contrast, when environmental conditions continually change, discordance arises between a species' genome and its environment, and a directional rather than a stabilizing selection takes place so that the average population genome moves to a new set point. Contemporary humans are genetically adapted to the environment of their ancestors (i.e., to the environment in which their ancestors survived and in which their genetic makeup was conditioned).

In essence, millions of years of evolution have shaped the need for nutrients, but our genes, controlling every function of the body, are basically the same as those of our ancestors *(1)*.

Profound environmental changes (e.g., in the diet and in other lifestyle conditions) began with the introduction of agriculture and animal husbandry about 10,000 yr ago, and have developed even faster in recent decades *(2)*. These changes have occurred too recently and rapidly on an evolutionary time scale for the human genome to adapt. The discordance between our ancient genetically determined biology and the patterns of

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nutrition, lifestyle, and cultural features in Western populations have been associated with the emergence of the so-called diseases of civilization.

Wild foods represented the major components of the diet of our ancestors. They substantially gathered in communities, with a social structure centered on "local traditional knowledge" *(3)*, including locally based diets. These included the collection and consumption of plants (gatherers), that through a long-term adaptation to the environment, have been locally growing for generations. In addition, the availability of small animal species and mini-livestock such as insects, rodents, snails, and frogs contributed to the food gathering process. Eating wild animals provided the most essential part of the nutrition (hunters).

In essence, these small human communities lived by moving from one selected area to another on the basis of seasonal, climatic, and geographic conditions, and of interactions and/or conflicts between communities. These migratory patterns were based on the search for wild animals that could be hunted and eaten. The local traditional knowledge that developed was substantially of "tribal" nature.

2. THE MEDITERRANEAN BASIN

In the Mediterranean area, especially in countries such as Greece, Southern Italy, and Spain, climatic and geographic conditions favored the settlement of local communities. The number of secluded areas separated by mountains, valleys, or hilly mountainous islands, favored the dissemination of local traditional knowledge, including food habits and consumptions, with some common features but also with some rather distinct characteristics. In the Mediterranean basin, plants are exposed to a stressful (pro-oxidant) environment and their adaptation in response to stress involves production of protective molecules such as polyphenols. These and other compounds have been shown to activate a group of components—the sirtuin family of deacetylases—that arose in primordial eukaryotes, possibly to help them cope with adverse conditions *(4)*. Sirtuins are found in plants, yeast, and animals and appear to be involved in slowing the aging process—implying a conserved underlying mechanism. The transfer of these compounds produced in response to stress signaling molecules from one species to another in their environment is the basis of the "xenohormesis hypothesis *(4)*." In this way, organisms can prepare in advance for a deteriorating environment. Whatever the conceptual basis of the above hypothesis may be, the intake of minor compounds produced in plants in response to stress is essential for human health and results in the transfer of the protective effects to our bodies. Research in this area will provide clues for improving our understanding of the mechanisms that activate the production of bioactive molecules in plants and in setting up strategies for the exploitation of their potential applications to optimized human nutrition.

3. BIOACTIVE AND HEALTH PROMOTING COMPOUNDS IN LOCALLY CONSUMED WILD MEDITERRANEAN PLANTS

A high consumption of fruits and vegetables is generally recommended as a health promoting dietary habit, being associated with greater intakes of micronutrients (such as minerals, vitamins, and bioactive compounds), of fiber, and of macronutrients with favorable nutritional properties, such as vegetable fats and proteins.

Epidemiological data and recent intervention studies indicate a beneficial effect of the Mediterranean diet on human health, especially on the prevalence of cardiovascular disease (CVD) *(5)*. The benefits of this diet can be attributed to the intake of compounds in plants that belong to three major groups: terpenoids, alkaloids, and phenolic compounds (mostly phenylpropanoids). Flavonoids—comprising mainly quercetin and kaempferol—and phenolic compounds—present in black tea, onions and apples—have been shown to exert risk lowering effects on mortality from coronary heart disease (CHD) *(6)*, possibly through potent antioxidant actions and activities on processes that are considered crucial in the pathology of chronic, aging-related diseases. However, very little is known about the current role of certain local Mediterranean plants, to a large extent still growing as wild or semi-wild plants, which are consumed on a less regular basis, and about their contribution to a healthy diet.

Data *(7)* were obtained in the European Union (EU) project "Local Food-nutraceuticals," aimed at assessing the ethnobotanical features as well as their biological activities of 127 locally consumed wild or semi-wild plants in three Mediterranean countries (i.e., Greece, Italy, and Spain). Biological activities were assessed on ethanolic extracts prepared from the dried plant material and covered a broad range of mechanisms considered crucial in the pathology of chronic, aging-related diseases. The polyphenol content of all extracts was determined and the extracts with greater concentrations of these compounds were tested. The assays included antioxidant tests (e.g., DPPH scavenging, prevention of oxyhaemoglobin bleaching, prevention of lipid peroxidation, and protection from DNA damage). In addition, enzyme inhibition tests (inhibition of xanthine oxidase, of myeloperoxidase-catalyzed guaiacol oxidation as well as the inhibition of acetylcholine esterase), and the inhibition of cytokine-induced cell activation and the antiproliferation potential were investigated. Finally, the antidiabetic activity (PPARγ) and one assay investigating the effect on mood disorder-related biochemical parameters (hSERT) were assessed. The assays revealed diverse biological effects for the tested extracts ranging from no activity to almost complete inhibition/activation. Moreover, the experimental matrix led to the identification of a subset of extracts—*(Berberis vulgaris*, *Reichardia picroides*, *Scandix australis*, *Satureja montana*, *Thymus piperella*, *Lythrum salicaria*, and *Vitis vinifera)*—from three plants consumed in all three regions in the Mediterranean basin that showed high activity in a broad range of assays. Therefore the in vitro observed modulations and effects exerted by extracts derived from local food plants suggest that these plants may contribute to the observed better aging of rural Mediterranean populations. As an example, extracts of these wild plants have been proven to exert antioxidant activities toward a variety of oxidants, including hypochlorous acid *(8)*. Moreover, *Cynara cardunculus* can restore vasomotion in the elderly *(9)*.

Another example of plant products that have been traditionally consumed by Mediterranean populations are olives and olive oil.

There are currently more than a dozen human studies on extra virgin olive oil, the majority of which demonstrates healthful activities on surrogate markers of cardiovascular disease.

4. FATS IN PLANTS IN THE MEDITERRANEAN AREA

Intakes of fats, mainly represented by fatty acids (FA), in the Mediterranean areas, in prehistorical times and to some extent also today, have been provided by the consumption

of the first type of vegetable fat in human diets (i.e., olive oil). In addition lipids present in plant foods of various nature (e.g., green vegetables, legumes, cereals, dry fruits) have contributed to the overall fat intake, that was altogether rather low.

Olive oil has been used throughout the millennia by ancient civilizations and presumably before the start of agriculture. Fossilized remains of the olive tree's ancestor were found in Italy, dating from 20 million yr ago, but cultivation probably did not occur in that area until the fifth century BC. Olives were first cultivated in the Eastern part of the Mediterranean, in the region known as the "fertile crescent," and moved westwards over the millennia. Beginning in 5000 BC and until 1400 BC, olive cultivation spread from Crete to Syria, Palestine, and Israel. Homer called it "liquid gold" and in ancient Greece, athletes ritually rubbed it all over their body. Olive oil has been more than mere food to the peoples of the Mediterranean: it has been medicinal, magical, an endless source of fascination and wonder and the fountain of great wealth and power, as evidenced by quotations in the literature from Mediterranean cultures (i.e., Israel, Greece, Rome).

Olive trees have an almost titanic resistance, a vital force that renders them nearly immortal. They keep growing despite harsh winters and burning summers, and bear nourishing and inspiring fruits despite truncation. The relevance of minor components in olives and olive oil have been previously mentioned.

5. CONTENTS AND HEALTH PROPERTIES OF SELECTED FA IN THE MEDITERRANEAN DIET: OLEIC ACID AND N-3 FA

5.1. Oleic Acid (OA)

This FA, the major component of olive oil and therefore the major FA in the typical diets of Mediterranean Countries, has some characteristic features. It has intermediate physicochemical properties, such as the "molecular shape" with a kink at the middle, with respect to saturated FA and polyunsaturated fatty acids (PUFAs). Therefore it represents a transition element in terms of melting point and of the features of biomembranes (e.g., fluidity) enriched in this FA. it is also the most abundant FA in most organisms, being efficiently biosynthesized. Vast literature has been produced on the health promoting effects monounsaturated FA, in particular oleic acid, which has been reviewed by Perez Jimenez *(10)*. The consumption of diets enriched in monounsaturated fat, replacing saturated FA, has been related to a lower rate of CHD, through decrease of plasma LDL-cholesterol levels, and also through nonlipid effects. In particular, other cardiovascular risk factors such as insulin resistance/impaired glucose metabolism, blood pressure, prevention of lipoprotein (LDL) oxidation, and modulation of coagulation, fibrinolysis and endothelial function have been attributed to this FA. The healthy effects of olive oil and of its minor components have been recently reviewed in detail *(11,12)*.

5.2. n-3 FA

Intake of long-chain (LC) PUFAs of the n-3 series were supported by fishing, a very ancient human activity. In fact, people have been fishing for about as long as they have been hunting. It has been even proposed that access to LC-PUFAs in the diet, typically present in fish, has been a promoting factor in the evolution of modern hominid brain *(13)*; most ancient civilizations developed around areas were fish was available for

consumption. However, judging from the literature of ancient civilizations (e.g., Greece), fish consumption was not a major component in the diet of populations living in the early historical eras in the Mediterranean. It is possible that the distant locations of human settlements from the coast favored the consumption of wild terrestrial animals and herds. Even today fish consumption, although relevant in the Mediterranean, is not generally higher than in Northern European countries (e.g., Norway). However, it should be pointed out that the Mediterranean diet is rather rich in α-linolenic acid (18:3 n-3, ALA) which are present in a large number of plant derived foods.

A number of studies have indeed shown that increments in the intakes of ALA, in the range of 1 to 2 g/d exert protective effects *(14–16)*. It is, however, still under debate as to whether the beneficial effects of ALA may be related to FA itself or to the LC-PUFA derivatives that may be generated from this FA, considering that production of the major 22 C compound, docosahexaenoic acid (DHA), has been shown to be not very efficient in terrestrial animals *(17)*. On the other hand, the advantage of ALA consumption rather than, or in addition to, that of the n-3 LC-PUFAs is that sources are practically inexhaustible resulting from the abundance of this FA in plant sources.

Data on the contents of ALA in wild plants and derived products consumed in the Mediterranean diet are not available. However, measurements of the contents in this FA expressed as g/portions in some major component of vegetables and legumes consumed in the Mediterranean provide the data shown in Table 1.

The absolute levels of ALA are rather low, but the relative richness of ALA in some selected vegetables and legumes, associated with the large size of the average portions and the high rates of consumption, result in appreciable levels of intakes. A regular portion of beans (about 120–140 g), provides significant amounts of ALA, almost 300 mg (i.e., about 15% of the recommended daily intakes [1–2 g]).

Even higher relative proportions of ALA are found in other edible plants growing in the Mediterranean basin, especially in the wild state *(16)*. Purslane, for instance, a plant that grows mainly in the wild state in southeastern countries such as Greece, has a high relative content of ALA *(19)*. Information on the general consumption rates (possibly high in certain communities) is limited.

5.3. Dry Fruits

The contents of ALA in certain dry fruits, notably walnuts and, to a significant extent, pistachios, are rather high (Table 1). One g of ALA is provided by the consumption of about 20 g of walnuts and about 400 g of pistachios. Walnuts were part of the diet of ancient populations. Though many historians pinpoint Persia as the country of the walnut's origin, archeological remains of walnuts were found as far east as the Himalayas and to the distant west and northwest of Persia into Turkey, Italy, and Switzerland. Dry fruits such as walnuts are therefore considered typical wild foods. Nut consumption has been shown to be beneficial for the cardioprotective effects *(20,21)*, possibly because this type of dry fruit provides very high amounts of ALA even in relatively low doses as a result of high fat contents and the high proportions of ALA (over 1 g in about 20 g of nuts corresponding to 4 nuts). This level of nut consumption over a 3-wk period results in significant elevations not only of ALA but also eicosapentaenoic acid (EPA) in whole blood lipids, in healthy subjects *(22)*. Other types of dry fruits are instead rather low in this omega 3 fatty acid.

Table 1
α-Linolenic Acid (ALA) Contents in Various "Wild" Foods (mg/100 g) and Percent Composition of ALA and LA on Total FA

Foods	*mg ALA/100 g*	*%ALA/FA*	*%LA/FA*
Legumes			
Beans	214.8	23.6	42.2
Lentils	110.0	12.9	49.7
Chickpeas	119.2	2.8	46.1
Red Lentils	103.0	11.3	48.5
Lupins	100.0	7.3	19.2
Leek	99.0	33.0	22.3*
Green lentils	57.0	7.5	46.8
Peas	47.4	5.4	45.9
Dry fruits			
South Italy Nuts	5280.0	14.6	57.3
Pistachio nuts	254.0	0.6	31.3
Pine seed (Naples)	158.1	0.6	49.9
Pine seed (Liguria)	100.0	0.6	50.3
Peanuts	20.5	0.1	29.0
Meats			
Hare	650.0	17.3	21.0§
Wild Rabbit	90.0	7.3	20.3
Processed deer's meat	33.9	1.4	7.2
Chamois	33.3	4.9	21.2
Frogs	29.5	5.2	19.8
Snails	28.3	4.0	23.3

*Data from USDA Food Composition Tables: *http://www.nal.usda*.
§Data from Danish Food Composition Tables: *http://www.foodcomp.dk/fcdb_search.asp*.

6. FATTY ACIDS IN WILD ANIMALS

Wild animals, hunted by prehistoric man, consumed diets based largely on grass and seeds and this resulted in lower fat contents in their meat. In addition, the intense muscular activity associated with continuous motion in the environment contributed to the low fat levels in these animals. As for the type of FA, they were mainly MUFAs and PUFAs, being associated with structural lipids in muscle membranes, with appreciable proportions of the n-3 FA.

Among wild animal foods still consumed today in the Mediterranean Countries, frogs and snails are of interest. Other types of wild animals that were eaten in prehistorical times are chamois, wild rabbit, and hares. Data on the FA composition of meat and other parts of these animals in prehistorical periods are obviously not available. However, laboratory measurements or data available from literature on available samples (Table 1) indicate that these foods provided appreciable amounts of ALA compared with other meats of the time, although they are not very relevant when compared with plant foods. Of particular interest is the contribution of hare's meat to ALA intakes, because 1 g is provided by about 150 g of food.

Wild foods in the diets of Mediterranean countries represent an excellent source of valuable nutrients from plant foods and wild animals. In addition to the richness in minerals, vitamins, and fibers that are typical of plants, especially those growing in the wilderness, the abundance of bioactive compounds produced in response to the stressful environment and transferred to animals and humans through the diet is very relevant from a nutrition and health standpoint. In addition, the relative abundance of selected minor, but biologically important FA, such as those of the n-3 series, both land (ALA) and sea based (LC-PUFAs) in wild foods in the Mediterranean area, and the relatively low contents in saturated FA in the same food items, contribute to their health protective effects.

Efforts should be made to increase awareness of the benefits of a traditional Mediterranean diet and both promote and sustain it's local cultural and biological diversity. Finally, the emerging knowledge about the properties of wild foods in terms of health promoting effects should encourage further research on the principles involved in these effects, particularly in human studies, and on their mechanisms of action. This with hopefully aid in search for and development of industrial strategies with applications aimed at optimizing human nutrition.

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33 Changing the Spanish-Mediterranean Diet

Effects on Plasma Lipids

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Abstract

The incidence of coronary heart disease has been traditionally low in Mediterranean countries. This has been linked to the Mediterranean-style diet and its effect, among others, on the plasma-lipid profile. Nevertheless, there are important differences in dietary patterns among different regions in the Mediterranean area. In general, the Spanish-Mediterranean diet is characterized by a relatively high content of bread, fish, green vegetables, eggs, olive oil, and red wine; however, variations among different regions may result in differences in the plasma-lipid profile. We have detected significant differences in high-density lipoprotein (HDL) cholesterol levels among regions in close proximity. These differences can be attributed to varying dietary patterns. Compared with subjects on a standard Spanish-Mediterranean diet, subjects on a high-meat diet showed a worse plasma-lipid profile. This did not happen in subjects on a high-fish diet. Neither ovo-lactovegetarian or lactovegetarian subjects presented better risk ratios (low-density lipoprotein cholesterol /HDL-cholesterol or Apo B/Apo A) than control subjects. In fact, in healthy subjects the suppression of all animal products in their diet (except eggs and dairies) did not result in a more favorable lipid profile, whereas increasing full-fat dairy-product intake in ovo-lactovegetarian subjects resulted in a net deterioration. This deterioration could be reverted by increasing physical exercise.

Key Words: Mediterranean diet; vegetarian diet; meat intake; fish intake; plasma lipids; HDL-cholesterol.

1. THE MEDITERRANEAN REGION AND ITS DIETS

The Mediterranean area is a broad geographical region, including at least 18 countries, that borders the Mediterranean Sea. The region can be roughly divided in two parts: the North-Mediterranean area located primarily in Europe and the South-Mediterranean area located in Africa. This chapter focuses primarily on the North-Mediterranean region, which has been the settlement of one of the oldest civilizations of the world. To a great extent, Western civilization and culture have their origins in this region. The geographical and climatological characteristics of the region coupled with a culture developed over several millennia have resulted in dietary patterns established via lifestlye among this population.

As in any other part of the world, the dietary practices in Mediterranean countries have been determined over the centuries by the local availability of specific foods. With these available foods, trial and error processes occurring over thousands of years have

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created an empirical body of food-related knowledge transmitted from one generation to the next. This knowledge has finally given rise to the development of a culinary culture. This culture allows the combination and preparation of available foods in ways that best preserve health (avoid disease) and guarantee lengthy and active lives. Types of foods available substantially differ among different regions even when they are in close proximity. Consequently, culinary cultures and dietary patterns may also greatly differ *(1)*.

In the past, the agricultural and farming activities of a particular region usually evolved in relation to its prevailing culinary culture and dietary habits. The reverse influence (i.e., of agriculture and farming on dietary habits) also occurred but was probably of lesser importance. It has only been recently (i.e., during the last century) that faster communication and transportation systems have made "foreign" foods easily available. This has led to recent changes in traditional dietary patterns. More recently, other circumstances such as commercial distribution of foods in supermarkets, decreasing use of the local marketplace, and the influence of mass media have determined additional and perhaps more important changes in dietary habits. These changes affect the population to a different extent. More affluent economies and younger subjects are probably more easily influenced.

Growing evidence demonstrates that the Mediterranean-style diet is beneficial to health. The evidence is stronger in terms of coronary heart disease (CHD), but also applies to stroke and some forms of cancer *(1–4)*. Large dietary variations occur in different countries. Ironically, a progressive departure from the traditional Mediterranean diet is being observed in Mediterranean countries *(5)*, whereas the diet is increasingly recognized as healthy and has therefore been adopted in non-Mediterranean, Western countries.

2. THE SPANISH-MEDITERRANEAN DIET

We should define the Spanish-Mediterranean-style diet as the usual diet of the inhabitants of Spain *(6)*. Nevertheless, geographical, economic, and agricultural differences have resulted in many different dietary practices in the country. This obviously precludes a single definition of the Spanish-Mediterranean diet. Nonetheless, one dietary pattern is common to all regions in the country. This traditional dietary pattern is composed of a cluster of basic foods that have been easily available in the region for centuries. The diet is high in fruits, green and root vegetables, bread, other forms of cereals, beans, nuts, and seeds of different types. A common and outstanding characteristic is the use of olive oil, which represents the more important source of fat in the diet. Animal-product intake includes eggs, dairy products, and poultry. There are significant variations in the intakes of fish, red meat (e.g., pork, cows, and lamb), and meat-derived products. In addition, red wine has traditionally been consumed. A main difference between the Spanish-Mediterranean diet and other Mediterranean diets is the lower intake of pasta and potatoes and the higher intake of bread, legumes, and fish *(6)*. Many of these components may have an effect on cardiovascular risk factors, particularly by influencing the plasma-lipid profile.

Differences and similarities among regions concern not only food content and composition but also cooking procedures, which may significantly affect the physiological actions of individual foods and their nutritional value. To give only one example, the nutritional/physiological consequence of ingesting a raw tomato onto which olive oil has been poured is probably different from the consequence of a cooked preparation *(7)*.

Furthermore, the consumption of raw green vegetables and fresh fruit is generally high, but there are important seasonal variations. Higher intakes of fresh fruit and green vegetables occur in spring and summer, whereas higher intakes of more caloric, boiled root vegetables, and seeds occur during cold weather. Obviously, the nutritional /physiological consequences concern not just calories or main nutrients but also vitamins, specific food components, and types of fat. These consequences are also related to the food preparation process (e.g., cooked combination of foods ready to be eaten) and not just from their constituents. These nutritional/physiological consequences are still largely unknown.

3. DIET AND LIPID PROFILE

Diet constitutes the best characterized exogenous factor among those that can influence the plasma-lipid profile. This is supported by results from ecological analyses, epidemiological surveys, prospective studies, animal experiments, clinical observations, controlled trials, and biochemical and nutritional studies *(8)*. It has also been the object of many comprehensive reviews *(9–14)*.

The most potent dietary influence is exerted by fats rich in saturated fatty acids and cholesterol. Individual variability in the response to dietary saturated fat and cholesterol is important, and it has a strong genetic component *(15)*. In addition to saturated fat and cholesterol, high intakes of animal protein may also play a major role in raising plasma cholesterol and low-density lipoproteins (LDL) *(16)*. Polyunsaturated fatty acids (PUFAs) of series ω-6 have a strong depressor effect on the cholesterolemia index, but they decrease both LDL and high-density lipoprotein (HDL) components; PUFAs of series ω-3 acids decrease cholesterol and triglycerides and may have a positive effect on HDL-cholesterol levels. Monounsaturated fatty acids (MUFAs), whose main exponent is the oleic acid found in olive oil, produce a neutral effect on cholesterolemia *(9)*. However, because of its low content of saturated fatty acids, olive oil decreases cholesterolemia when substituted for saturated fats in the diet. Several researchers have demonstrated that olive oil is able to increase HDL-cholesterol plasma concentration, which is considered beneficial in view of the inverse relation of these lipoproteins with cardiovascular risk *(17)*. This oil may have other beneficial effects, too, particularly those linked to the high content of antioxidant agents *(18)*. Carbohydrates (particularly starch and refined carbohydrates) are neutral in respect to LDL-cholesterol levels, but they raise triglyceride concentrations and may reduce the mean particle size of LDL *(19,20)*. This, together with low HDL-cholesterol level, is a characteristic feature of the atherogenic lipoprotein profile.

Some dietary trials designed to reduce blood cholesterol by a low-cholesterol, low-saturated fat, high-polyunsaturated-fat content failed to demonstrate an overall clinical benefit for the dieters. In contrast, other studies not primarily intended to reduce blood cholesterol have successfully prevented the recurrence of cardiovascular events. The main dietary characteristic in these studies was an enhanced intake of marine or plant ω-3 fatty acids *(21–23)*. In addition to cholesterol, fat, proteins, or carbohydrates, other components of the diet, such as soluble fiber, alcohol, antioxidants, subcategories of fatty acids, vitamins particularly of the B group, vegetable proteins, phytosterols, flavonoids, and many other dietary components may also be influential. In fact, other diets showing positive effects on cardiovascular risk were high in fresh fruits and

vegetables, legumes, and cereals containing large amounts of fiber *(12,13)*. As repeatedly stated *(24)*, the quality and biological impact of foods can no longer be properly assessed only in terms of proteins, complex and simple carbohydrates, or a few types of fat. Many other dietary components are also biologically active, and there is a place for epidemiological and clinical investigation of the effects of particular foods (not only some of their components) on risk factors for chronic disease. In addition, one area of nutritional research that is almost untouched is the interaction of nutrients. A new trend of studying dietary patterns rather than specific dietary components may help to elucidate observed effects *(25,26)*.

4. REGIONAL DIFFERENCES IN HDL-CHOLESTEROL LEVELS: DOES DIET PLAY A ROLE?

HDL-cholesterol levels in a representative sample of healthy subjects (blood donors) from different geographical regions in close proximity was measured. Surprisingly, we found statistically (and biologically) significant differences in the inhabitants of these regions *(27)*. All sample regions were within the Granada province in southeastern Spain. The regions studied and listed in Fig. 1 were the littoral with several medium size villages of up to 60,000 inhabitants, the city (Granada) with some 250,000 inhabitants, an industrial suburban area integrated by small villages of up to 20,000 inhabitants around the city and totaling another 250,000 inhabitants, the fertile valley containing the only route linking the coast with the metropolitan area, two agricultural regions located in the inner country and integrated by rather small villages totaling another 200,000 inhabitants, and a mountain agricultural zone.

The entire region is typically Mediterranean. In addition, southern Spain is the largest producer of olive oil in the world. The littoral presents two peculiarities. On the one hand, it has a specific sea ecosystem influenced by cold-water currents coming from the Atlantic Ocean. For that reason, some types of small, fatty fish are particularly abundant and have been traditionally consumed and exported from the coast to the capital through the only road linking both regions. In the coast and in the valley, the temperate climate allows green-vegetable, fruit, and tomato (intensive) production throughout the year. The littoral is a main provider of tomatoes for non-Mediterranean Europe. In contrast, some regions in the interior, particularly the mountainous areas, are dry and present poor soils for agricultural production. Rural areas in the province have an agriculturally based economy.

Higher HDL-cholesterol levels were observed in the villages situated along the coast and along the only road linking the littoral to the city. Intermediate levels were found in two different agricultural areas inside the region. Lower levels were found in the rather industrial villages around the city, the city itself, and the poor, rural, mountain area (*see* Fig. 1). We do not have any explanation for these differences, but obviously important variations in lifestyle habits do exist among these regions. Probably the more important one concerns dietary habits.

We consequently investigated meal habits in the different regions, and we found both a series of common characteristics and several important differences. The qualitative-assessment results of these dietary peculiarities are as follows:

- Common characteristics: (i) similar high intakes of bread, olive-oil, green vegetables, fruit, eggs, wine, and beer; (ii) similar low intakes of pasta; and (iii) similar very low

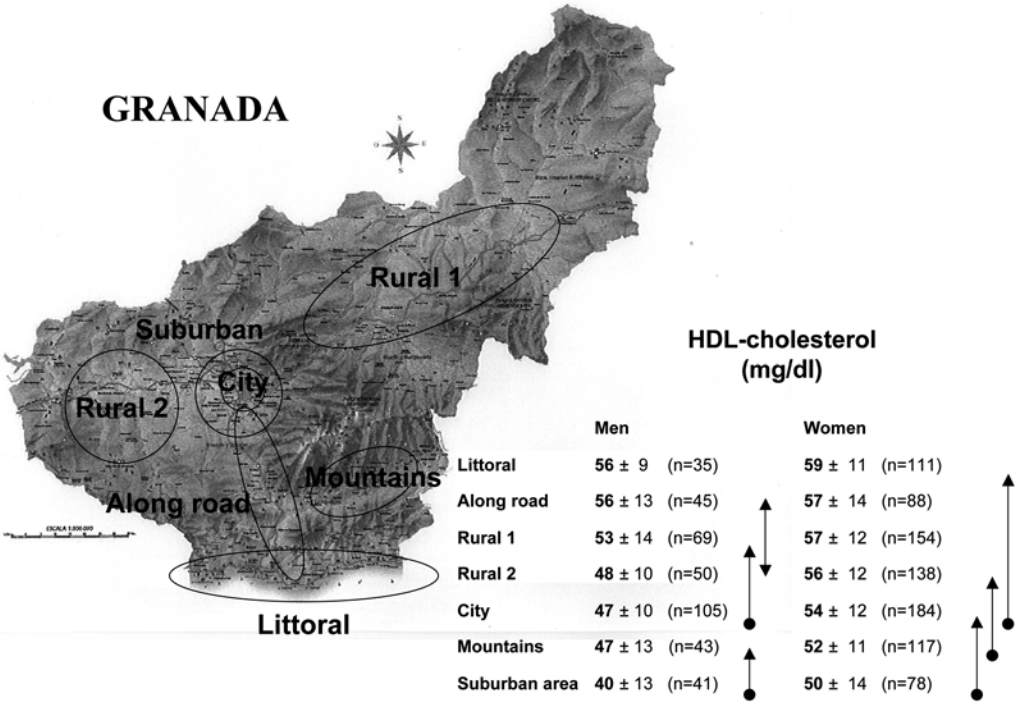

Fig. 1. HDL-cholesterol levels measured in healthy blood donors living in several areas of the region. These areas are: the littoral, valley from the littoral to the city, two different rural areas, a mountain area, the city and a suburban area around the city. Arrows indicate statistical significant differences between values.

intakes of cow meat, lamb (almost absent), butter (almost absent), hydrogenated fat, non-olive oil, and fast foods.

- Main differences among regions were as follows: (i) Fish intake: higher in littoral areas, city, and the villages along the road from the littoral to the city (6–7 servings/wk); lower in rural areas (1–2 servings/mo.). (ii) Meat intake (poultry and pork): higher in suburban and urban areas; lower in littoral areas. (iii) Pork-derived, fatty-product intake: higher in rural areas; lower in littoral areas. (iv) Homegrown-product (including eggs) intake: higher in rural areas; lower in urban and suburban areas. (v) Pastry intake: higher in the city; lower in rural areas. (vi) Root vegetables and seeds: higher in rural areas and suburban villages; lower in littoral areas.

Differences in dietary practices in areas that are relatively close can be explained by food availability (local production of foods, soil productivity, and transport facilities). For instance, in some rural (mountain) areas, intake of meat-derived products (particularly pork) was high, but it mostly included products (e.g., sausages, bacon) with a high-fat content. Fish was virtually absent, historically because of transport problems and more recently because of lack of habit. In contrast, the coast is both a traditional fishing area and a fertile zone of subtropical climate. Green vegetables and virtually all kinds of fruit are produced without interruption throughout the year and exported. Fish and agricultural products are transported day and night over the only road linking the coast and the

Table 1
Plasma Lipid Profile of Healthy Men With Different Dietary Patterns Superimposed to a Spanish Mediterranean Diet

	Control n = *18*	*Fish* n = *9*	*Meat* n = *15*	*OLV* n = *16*	*LV* n = *9*
Cholesterol	185 ± 5	210 ± 16	222 ± 7^{C,L}	200 ± 8	148 ± 6^{C,F,O}
LDL-cholesterol	113 ± 3	136 ± 15	146 ± 6^{C,L}	127 ± 8	93 ± 4
HDL-cholesterol	52 ± 2	51 ± 3	48 ± 2	56 ± 3	37 ± 3^{C,F,O}
LDL-/HDL-chol	2.2 ± 0.1	2.8 ± 0.4	3.1 ± 0.2^{C}	2.4 ± 0.2	2.6 ± 0.3
Triglycerides	95 ± 7	122 ± 22	136 ± 15^{C,O}	83 ± 8	90 ± 8
Apo B	119 ± 5	148 ± 11^{C}	154 ± 6^{C,L}	125 ± 8^{M,F}	87 ± 5^{M,F}
Apo A_1	266 ± 8	279 ± 14	263 ± 14	270 ± 12	215 ± 15
Apo B/Apo A_1	0.4 ± 0.02	0.5 ± 0.03	0.7 ± 0.06	0.5 ± 0.04	0.4 ± 0.04

Notes: Control, subjects on a standard mixed diet; Fish, fish eaters; Meat, meat eaters; OLV, ovolactovegetarian subjects; LV, lactovegetarian subjects.

Results are expressed as mean ± SEM. Units are in mg/dL.^{C,F,M,O,L} $p < 0.05$ vs control, fish, meat, OLV, LV.

city. These peculiarities are reflected in the traditional cuisine of the region, which is a clear reflection of the local availability of food products.

Obviously, these dietary characteristics may influence the plasma-lipid profile and may explain the observed differences in HDL-cholesterol levels, although other factors can also be influential *(28)*. Differences in HDL-cholesterol levels in the region correspond to the widespread idea of regional differences in cardiovascular disease (CVD) *(29–31)*. All the explored regions had a dietary pattern with the characteristics of the Mediterranean-style diets, but the healthier pattern was observed in the littoral (and its area of influence) and more affluent rural areas. It is precisely in those regions where higher HDL-cholesterol levels were observed. The higher intake of animal products (except fish) was observed in suburban villages. It is precisely in that region where lower HDL-cholesterol levels were detected.

5. CHANGES IN THE SPANISH-MEDITERRANEAN DIETARY PATTERN

In order to study the influence of different dietary patterns superimposed on the traditional Spanish-Mediterranean diet, we looked for healthy, nonobese subjects with diets particularly rich in meat and meat-derived products or fish, or containing no meat or fish (i.e., ovo-lactovegetarian or lactovegetarian subjects); we also studied a control group. Meat or fish eaters were recruited among the owners or employees of butcheries and fish shops. Subjects in the meat-eater group ate more than 10 servings/wk of meat or meat-derived products. Subjects in the fish-eater group ate more than 7 servings/wk of fish. Blood collections were obtained after an overnight fast. The plasma-lipid profiles of these subject groups are presented in Tables 1 and 2 *(27)*. A basic Spanish-Mediterranean dietary pattern was preserved in all subjects.

As can be seen, subjects with high meat or meat-derived product intakes presented the worst plasma-lipid profile with increased levels of total cholesterol, triglycerides, LDL-cholesterol, apo-B, and LDL-/HDL-cholesterol ratios. Compared with the control

Table 2
Plasma Lipid Profile of Healthy Women With Different Dietary Patterns Superimposed to a Spanish Mediterranean Diet

	Control n = *27*	*Fish* n = *9*	*Meat* n = *8*	*OLV* n = *9*	*LV* n = *9*
Cholesterol	187 ± 5	200 ± 9	221 ± 8^{C,F,O,L}	197 ± 10	149 ± 12^{C,F,M,O}
LDL-cholesterol	112 ± 4	125 ± 10	147 ± 12^{F,L}	118 ± 6	87 ± 6^{C,M,F,O}
HDL-cholesterol	62 ± 2	59 ± 4	52 ± 3	64 ± 5	48 ± 3^{C,O}
LDL- / HDL-chol	1.9 ± 0.1	2.2 ± 0.2	3 ± 0.2^{C,F,O,L}	1.8 ± 0.1	1.9 ± 0.2
Triglycerides	66 ± 3	77 ± 9	108 ± 10^{C,F,O,L}	69 ± 5	69 ± 5
Apo B	106 ± 4	133 ± 9^{C}	137 ± 12^{C}	106 ± 7^{F}	75 ± 5^{C,M,F,O}
Apo A_1	269 ± 4	292 ± 20	285 ± 8	302 ± 15	242 ± 15^{O}
Apo B/Apo A_1	0.4 ± 0.02	0.5 ± 0.04	0.5 ± 0.04	0.4 ± 0.03	0.3 ± 0.02^{M,F}

Notes: Control, subjects on a standard mixed diet; Fish, fish eaters; Meat, meat eaters; OLV, ovolactovegetarian subjects; LV, lactovegetarian subjects.

Results are expressed as mean ± SEM. Units are in mg/dL.^{C,F,M,O,L} $p < 0.05$ vs Control, Fish, Meat, OLV, LV.

group, subjects eating high-fish diets did not present significant differences in their lipid profiles except for slightly higher apo-B levels. Ovo-lactovegetarian subjects did not have a better profile than subjects on a common Spanish-Mediterranean diet. Lacto-vegetarian subjects showed decreased LDL- and HDL-cholesterol levels as well as lower apo-B and apo-A levels. Consequently, their risk profiles expressed as risk ratios were no better than the risk profiles presented by the control or ovo-lactovegetarian subjects.

6. ELIMINATION OF MEAT AND FISH FROM THE SPANISH-MEDITERRANEAN DIET

It was rather surprising that ovo-lactovegetarian subjects did not present a better plasma-lipid profile than subjects on a common Spanish-Mediterranean diet. Consequently, in subjects on a common local diet, we investigated the consequences of suppressing all animal products except eggs and dairy products. We selected a group of highly motivated subjects who would not receive any compensation (economic or other) for their participation. The decision to adhere to a vegetarian diet was taken by the subjects independent of their participation in the study. Subjects were evaluated twice: before and 2 mo after dietary manipulation. At study entry, their diets included meat and meat-derived products at an average rate of 6 times/wk; chicken and pig had a much greater predominance. Fatty fish, mostly fried, was eaten 3 to 4 times/wk *(32)*.

After 2 mo of meat and fish suppression, the subjects presented lower intake of proteins, saturated fat, and cholesterol (Table 3). Total carbohydrate intake was not modified, but the percent contribution to total caloric intake increased. Interestingly, oleic-acid intake and its contribution to total caloric intake also decreased (Table 4). This was explained by the decrease in fried foods (fish and meat), which were substituted with scrambled eggs with various legumes (also typical of Spanish cuisine). After this dietary intervention, cholesterol levels decreased not in the LDL fraction but in the HDL fraction (Table 4). Possible explanations for this unexpected effect were the decrease in

Table 3
Daily Intakes (Mean ± SEM) of Several Dietary Components Before and After the Elimination of All Animal Products (Except Eggs and Dairy Products) from a Mixed Mediterranean Diet in 14 Healthy Young Subjects

	Before	*After*
Energy intake (kcal)	2970 ± 244	2590 ± 254*
Proteins (g)	98.3 ± 7.4 (13.2 ± 0.5%)	77.4 ± 8.1* (11.3 ± 0.4%)*
Carbohydrates (g)	391 ± 41 (49.6 ± 2.3%)	379 ± 43 (55.2 ± 1.6%)*
Fat (g)	118 ± 9 (35.8 ± 2.3%)	93 ± 9* (31.7 ± 1.5%)
Saturated (g)	43.8 ± 5.0 (13.2 ± 0.9%)	34.8 ± 4.5* (11.8 ± 1.2%)*
Monounsaturated (g)	56.1 ± 3.1 (17.2 ± 2.3%)	42.1 ± 4.2* (14.6 ± 1.0%)*
Polyunsaturated (g)	17.2 ± 2.3 (5.0 ± 0.4%)	15.2 ± 2.8 (5.1 ± 0.6%)
P/S ratio	0.40 ± 0.03	0.44 ± 0.05
Cholesterol (g)	0.47 ± 0.06	0.28 ± 0.03*
Fiber (g)	15 ± 2	16 ± 3

*Significant change ($p < 0.05$) after versus before. Percentages of total caloric intakes are indicated in brackets.

Table 4
Plasma Lipid Profile (Mean ± SEM) and 95% Confidence Intervals, in 14 Healthy Young Subjects Before and After the Elimination of All Animal Products (Except Eggs and Dairy Products) from a Mixed Mediterranean Diet

	Before		*After*	
	Mean ± SEM	*Confidence intervals*	*Mean ± SEM*	*Confidence intervals*
Total cholesterol	175 ± 5	164–186	166 ± 5*	155 – 177
LDL-cholesterol	97 ± 6	85–109	94 ± 5	83 – 105
HDL-cholesterol	64 ± 3	57–69	57 ± 3*	51 – 64
LDL-/HDL-cholesterol ratio	1.57 ± 0.11	1.33–1.82	1.69 ± 0.12	1.42 – 1.96
Triglycerides	61 ± 4	51–71	70 ± 9	50 – 89
Apo B	64 ± 4	54–73	60 ± 3	54 – 66
Apo A_1	132 ± 15	111–150	140 ± 12	120 – 151
Apo B/Apo A_1 ratio	0.52 ± 0.04	0.43–0.62	0.46 ± 0.04	0.38 – 0.54

*Significant change ($p < 0.05$) after the second study period. Units are in mg/dL.

oleic acid intake, the higher contribution of carbohydrates to caloric intake (although plasma triglycerides were not increased), or the lower fat intake *(32)*. We could conclude that eliminating all animal products except eggs and dairy products from the current Spanish-Mediterranean diet had no beneficial effects on the plasma-lipid profile.

7. CONSEQUENCES OF INCREASING DAIRY PRODUCT INTAKE

Vegetarian subjects present low fat and low animal-protein intakes. For most of them, dairy products represent a major source of cholesterol, saturated fat, and animal protein in their diet *(33)*. In fact, in vegetarian subjects LDL-cholesterol concentrations

are correlated with dairy products consumption *(34)*. In these subjects the increased intake of whole-fat dairy products may negatively affect plasma-lipid profiles. We then investigated in ovo-lactovegetarian and nonvegetarian control subjects whether an increase in whole-fat dairy-product intake effectively modified the plasma-lipid profile *(35)*. This diet manipulation increased energy, protein, fat (resulting from increased saturated fat), and cholesterol intake (Table 5). In nonvegetarian subjects, protein and saturated-fat intake also increased. In this group, the results of other dietary intakes were masked by differences in baseline values (Table 5). At baseline, vegetarian subjects, compared with nonvegetarian, only presented significant lower apo-B levels (Table 6). After the first 2 mo of dietary manipulation, the evolution of the plasma-lipid profile differed in both groups. In the vegetarian group, the increase in dairy-product intake induced significant increases in LDL-cholesterol and apo-B levels as well as in LDL-/HDL-cholesterol and apo-B/apo-A_1 ratios. In the non-vegetarian control group, the diet manipulation did not significantly affect the plasma-lipid profile (Table 6). Milk fat contains some of the most commonly consumed saturated fatty acids, such as lauric (C12:0) and myristic (C14:0) acids, which have a more pronounced influence on plasma cholesterol; in addition, fatty dairy products are also rich in cholesterol, which contributes to increased LDL-cholesterol levels*(9,36)*. Our results are concordant with this hypothesis.

8. INFLUENCE OF PHYSICAL EXERCISE

Diet and physical exercise interact in the development and prevention of ischemic heart disease. Both factors affect the plasma-lipid profile. A physiological means of influencing diet-induced modifications of the plasma-lipid profile is physical exercise *(37,38)*. Physical activity favorably influences all three components of the atherogenic lipoprotein phenotype: the HDL-cholesterol concentration may increase, LDL-cholesterol may decrease, and serum triglycerides can also be reduced *(39,40)*. Therefore, the effects of dietary-induced hypercholesterolemia may be attenuated by endurance exercise *(39,40)*. To test this hypothesis in the above mentioned subjects, a 2-mo exercise program was proposed. The exercise program had to be practiced in addition to subjects' daily physical and training activities and consisted of a session of aerobic exercise performed once every 2 d. Each session consisted of running or cycling for 30 to 45 minutes at a minimal intensity of 60% maximal oxygen consumption ($VO_{2\ max}$). These training sessions were done in the laboratory under the presence of one of the investigators. Dietary patterns (Table 5) were not modified during this period *(35)*.

The increased physical activity had a positive influence on both groups (Table 6). In vegetarian subjects, physical exercise reduced LDL-cholesterol and apo-B levels, and significantly increased HDL-cholesterol levels, although this was not accompanied with a similar effect on apo-A_1 plasma concentration. Consequently, LDL-/HDL-cholesterol, but not apo-B/apo-A_1 ratios, returned to baseline values (Table 6). In the non-vegetarian control group, the training program also induced significant reductions in LDL-cholesterol and apo-B levels *(35)*. Consequently, regular physical exercise may offset the effect of high intakes of cholesterol, saturated fat, or other dietary components on the plasma-lipid profile.

Table 5
Mean ± SD Daily Intakes of Several Dietary Components Evaluated at Baseline, After 2 Mo of Supplemented Dairy Product Intake (Diet) and After 2 Additional Mo of Enhanced Training (Diet + Exercise) in 14 Healthy Ovo-Lactovegetarian and 10 Nonvegetarian Control Subjects

	Ovo-lactovegetarian subjects			*Non-vegetarian control subjects*		
	Baseline	*Diet*	*Diet + Exercise*	*Baseline*	*Diet*	*Diet + Exercise*
Energy intake (kcal)	3035 ± 754	3461 ± 798*	3542 ± 569*	3598 ± 900	3860 ± 894	3941 ± 866*
Proteins (g)	84 ± 25	120 ± 23*	114 ± 17*	124 ± 25§	145 ± 17*§	154 ± 18*§
Carbohydrates (g)	413 ± 126	430 ± 150	439 ± 111*	441 ± 113	425 ± 111*	432 ± 120
Fat (g)	103 ± 34	142 ± 39*	150 ± 27*	164 ± 58§	175 ± 48	173 ± 51
Saturated (g)	31 ± 14	63 ± 20*	70 ± 14*	54 ± 25§	65 ± 22*	75 ± 22*
Monounsaturated (g)	51 ± 17	57 ± 19	58 ± 14	86 ± 36§	90 ± 32§	75 ± 37
Polyunsaturated (g)	20 ± 11	22 ± 13	22 ± 11	22 ± 7	21 ± 6	23 ± 6
P/S ratio	0.67 ± 0.36	0.37 ± 0.24*	0.33 ± 0.25*	0.44 ± 0.13	0.34 ± 0.10*	0.33 ± 0.13*
Cholesterol (g)	257 ± 106	423 ± 116*	441 ± 82*	839 ± 557§	906 ± 355§	895 ± 468§
Fiber (g)	22 ± 9	24 ± 8	26 ± 10	17 ± 9	16 ± 6	16 ± 8

*$p < 0.05$ vs Baseline.
¶$p < 0.05$ vs Diet.
§$p < 0.05$ vs ovo-lactovegetarian subjects.

Table 6
Plasma Lipid Profile Values (Mean ± SD) Measured at Baseline, After 2 Mo of Supplemented Dairy Product Intake (Diet) and After 2 Additional Mo of Enhanced Training (Diet + Exercise), in Healthy Ovo-Lactovegetarian (n = 14) and Non-Vegetarian (n = 10) Control Subjects Units are in mg/dL

	Ovo-lactovegetarian subjects			*Non-vegetarian control subjects*		
	Baseline	*Diet*	*Diet + Exercise*	*Baseline*	*Diet*	*Diet + Exercise*
Total cholesterol	161 ± 18	167 ± 20	169 ± 23*	171 ± 17	171 ± 30	164 ± 21
LDL-cholesterol	89 ± 17	97 ± 16*	93 ± 20	97 ± 13	95 ± 20	89 ± 12*
HDL-cholesterol	56 ± 10	55 ± 14	68 ± 12*¶	61 ± 15	61 ± 16	62 ± 16
LDL-/HDL-cholesterol	1.65 ± 0.47	1.94 ± 0.68*	1.60 ± 0.50¶	1.67 ± 0.41	1.67 ± 0.57	1.51 ± 0.37
Triglycerides	80 ± 37	77 ± 26	75 ± 20	63 ± 16	74 ± 29	64 ± 13
Apo B	68 ± 16	75 ± 15*	68 ± 13¶	85 ± 14§	86 ± 18	78 ± 12*
Apo A_1	140 ± 41	132 ± 49	125 ± 34	153 ± 28	164 ± 47	138 ± 41
Apo B/Apo A_1	0.46 ± 0.15	0.66 ± 0.30*	0.57 ± 0.11	0.58 ± 0.16	0.56 ± 0.16	0.64 ± 0.28

*$p < 0.05$ vs Baseline.
¶$p < 0.05$ vs Diet.
§$p < 0.05$ vs ovo-lactovegetarian subjects.

9. CONCLUSION

The Mediterranean-style diet has been shown to be protective for coronary artery disease, but there are important differences in dietary patterns among different regions. These differences may influence the plasma-lipid profile even when a common Mediterranean pattern is maintained. High intakes of animal products (except fish) have a negative influence. This influence is probably worse in other Western diets devoid of some basic characteristics of the Mediterranean diet. Physical exercise may partly reverse diet-induced negative effects on the plasma-lipid profile.

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34 Lebanese Traditional Diets and Health Effects

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Abstract

Lebanon is a highly urbanized Mediterranean country, characterized by its healthy traditional cuisine. With some exceptions related to sugar, sweeteners, and cereals, Lebanese food consumption is comparable with many northern Mediterranean countries. It is a collection of minimally processed vegetarian recipes, in addition to an abundance of fruits, vegetables, cereals, legumes, and nuts. Olive oil is the principle fat used, replacing other sources, in addition to many other ingredients including wild edible plants, lemon, garlic and mint.

With modernization and development, consumer tastes and demands have had implications on the traditional Lebanese foods and dietary habits. Some changes occurred to the ingredients used and to the percent contribution of macronutrients to energy intake. Recent studies on food consumption patterns of the Lebanese young and adult population showed a shift in the food consumed toward increased intake of fat, milk, and animal protein and decreased intake of whole wheat bread and cereals. It seems that the Lebanese Mediterranean diet is converging with a pattern high in saturated fat, sugar, and refined foods and is low in fiber. Such a Westernized dietary pattern is associated with the increased risk of non communicable diseases such as obesity, cardiovascular disease, diabetes, and hypertension.

Although the present Lebanese dietary habit has retained many of its Mediterranean characteristics, however, a duality is appearing between modernization and the strong adherence to culture and tradition.

Key Words: Lebanon; traditional diet; health.

1. LEBANESE TRADITIONAL DIETS

Lebanon is a Mediterranean country with a territory of 10,452 km^2 and an estimated population of about 4 million and with 40% of its population living in the capital Beirut, considered to be the melting pot of the country. The Lebanese traditional diet constitutes an intangible cultural heritage and has been described as a healthy dietary pattern. People in Lebanon recognize their cuisine as distinctive and expressive of their identity. It is transmitted from mother to daughter, generation after generation. The Lebanese cuisine draws on a rich culinary history, and reflects Lebanon's unique interaction with the Babylonians, Phoenicians, Egyptians, Greeks, Romans, Persians, Byzantines, and Turks. Details of ingredients, seasoning and cooking procedures all constitute inseparable components of the Lebanese dietary traditions. The literature describes the Lebanese diet of the early 1960s by the following broad characteristics: an abundance of plant food

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(e.g., fruit, vegetables, breads, other forms of cereals, potatoes, legumes, nuts and seeds); minimally processed fresh fruit as the typical daily dessert with sweets containing concentrated sugars or honey consumed a few times per week; olive oil as the principle source of fat, replacing other oils, butter and margarines; dairy products (e.g., mainly cheese and yogurt) consumed daily in low to moderate amounts; wild edible plants used in many of the traditional dishes; fish, poultry and red meat consumed in low amounts; and wine consumed in low to moderate amounts *(1–3)*. Thus, the Lebanese traditional diet seemed to include many items typical of the mediterranean Middle Eastern diet, renowned for its positive impact on health.

A Mediterranean diet *per se* is difficult to define. It is a loose term that describes the eating practices of the people in this region. Around 18 countries reside on the coast of the Mediterranean Sea, including Spain, France, Italy, Croatia, Bosnia, Albania, Greece, Turkey, Syria, Lebanon, Palestine, Egypt, Libya, Tunisia, Algeria, and Morocco, in addition to the island countries Malta and Cyprus *(4)*.

Interest in the diet of the Mediterranean region has risen from large scale studies, demonstrating its positive effect on longevity and prevention of morbidity *(5–7)*. These studies provided supportive evidence indicating that following a mediterranean diet, characterized by a high ratio of monounsaturated to saturated fat, high consumption of legumes, moderate intake of fish, low-to-moderate intake of dairy products (mostly in the form of cheese and yogurt), moderate intake of ethanol, primarily from wine, and a low intake of saturated fats, meat, and poultry, is an effective measure for protection against mortality, especially that arising from chronic diet-related diseases, such as cardiovascular diseases and cancer *(4–8)*. In the Lyon heart study, patients who suffered from myocardial infarction (MI) experienced a reduction in cardiac events and deaths of close to 70%, following a Mediterranean diet regimen *(9)*. Important reductions in the relative risks of acute MI were also observed in association with consumption of olive oil, fiber, fruits, vegetables, fish, and alcohol. Conversely, a positive association was reported between high consumption of meat, refined cereals, and high glycemic load foods (e.g., white bread, pasta, and white rice) and MI *(10)*.

Thus, the traditional Lebanese diet, as described by early researchers, acquired its healthy Mediterranean characteristic from its diversity of food, which appear to have provided healthy ingredients. Early national studies that examined the nutritional status of the Lebanese population did not identify any deficiencies in essential nutrients *(3,11)*. From the foods described in these studies, it is possible to surmise that the traditional Lebanese diet had the macro- and micronutrients as well as other important ingredients, now discovered to be protective against noncommunicable diseases, such as ω-3 fatty acids, fiber, phenolic compounds, terpenes, organosulfides, and indoles. Of the foods consumed, nuts and seeds, as well as dark green leafy vegetables, such as spinach and purslane provided ω-3 fatty acids, associated with reduced risk of heart disease, depression, and inflammatory conditions *(12)*. Fruits, vegetables, pulses, legumes, and nuts have provided the fiber, protective against constipation, cancer (especially colon cancer), heart disease, and diabetes *(13)*. Jew's mallow provided the soluble dietary fiber, associated with decreased serum and cholesterol concentrations as well as increased excretion of bile acids and neutral sterols *(14)*. Olive oil, parsley, thyme, onions, tea, beans, nuts, tomato, citrus fruits, celery, wheat, lentils, chickpeas, and other legumes provided the phenolic compounds, reported to possess antioxidant and anticancer

potentials, and to be protective against many inflammatory conditions, especially when isolated from olive oil *(15)*. Dark green leafy vegetables, citrus fruits, and legumes provided the isoprenoids, which may have acted as anti-inflammatory, antiproliferative, and anticancerous agents *(16)*. Garlic and onions, which are used routinely as essential flavoring ingredients in the Lebanese diet, provided organosulfides, recently reported as potential chemopreventive agents with antioxidant properties, protecting against coronary heart diseases, as well as possessing antimicrobial and antimutagenic activities *(17)*.

Comparing the traditional Lebanese dietary habits with the recent one revealed a remarkable preservation of some traditional foods, with some modern ingredients added in food preparation. As reported by Noah and Truswell *(4)*, white flour, white rice, and burghul are now commonly used by people from many Mediterranean countries, including Lebanon, instead of whole wheat items. Potato is eaten as part of different dishes, not as a staple food, and vegetables dishes, prepared with olive oil and tomato puree, are being served either cold without meat or hot with meat. The main vegetables currently consumed in Lebanon are the eggplant, broad and green beans, okra, zucchini, and vine leaves. Melokhia (Chorchorus olitorius or Jews' mallow) and stuffed vine leaves continue to be consumed by the Lebanese, as a distinctive feature of their diet. Cherries and oranges are among the most commonly consumed types of fruits. Consumption of dried pulses is still high to moderate. Also noted is a high consumption of pistachio nuts, used in traditional sweets, and sesame seeds, used to make tahini (sesame seed paste).

These observations were also confirmed by other nutritional studies in Lebanon, which reported a shift in food consumption toward increased intake of fat, milk, and animal protein and a decrease in the intake of non-refined carbohydrates, in particular bread and cereal *(18)*. The contribution of bread and other cereal products to daily energy intake dropped from 58 to 36% between 1964 and 1998, and the contribution of total carbohydrates to daily energy intake decreased from 64.8% between 1961 and 1963 to 52.9% in 1998, whereas the contribution of fat increased from 24 to 34.3% during the same period.

Other studies showed that the Lebanese diet still retained many of its traditional characteristics. Calculations from FAOSTAT database on Lebanese food supply, by Mouawad H. *(19)*, showed that, in Lebanon, neither tradition nor modernity is the exclusive prerogative of cities or villages. Cereals remained the first source of calories in the Lebanese diet from years 1961 to 2001. Also noted was a beneficial increase in the supply of fruits, vegetables, and pulses. In 1961, Lebanon was found to occupy the first position in terms of fruits and vegetables supply per capita. In 2001, Lebanon occupied the second position after the Greece. Mouawad *(19)* also noted that total fats' supply level in Lebanon doubled during a period of 40 yr. Mouawad *(19)* concluded that, despite maintaining some of their traditional aspects, the Lebanese dietary habits, including food practices and food products, are being influenced by modernity. Consequences of modernity on food practices were reported as follows: disintegration of meals, increase of nibbling, introduction of the food in working places, development of the fast food, and decrease of time dedicated to the cooking *(20–22)*. As for the consequences of modernity on food products, they were reported as follows: more imported products, more industrialized products, more meat-based products, fewer staple foods, more frozen products, more low fat products, more exotic products, more sugar, and lesser influence of the seasons.

The study of Nasreddine et al, *(23)* showed that adults in Beirut consumed a diet characterized by an acceptable level of intake of fruits and vegetables. The consumption of fresh fruits and vegetables combined was estimated to be 367 g/d, thus approaching the World Health Organization and Food and Agriculture Organization minimum recommended value of 400 g/d *(24)*. Adequate consumption of fruits and vegetables is associated with significantly lower risk of cardiovascular diseases, obesity, diabetes, and certain types of cancer. Their protective effects are probably mediated through numerous beneficial nutrients including antioxidants, vitamins, minerals, phytochemicals, fiber, and plant protein *(25)*. A low consumption of fish was also noted in this study, with an average value of 19.7 g/d, with 73.6% of the subjects consuming less than the recommended 2 servings of fish/wk (180 g) and 64.6% less than 1 serving/wk. There is strong evidence for the inverse correlation between fish consumption and the risk of death from CHD *(26)*. Protective effects are most likely related to the cardiovascular benefits of ω-3 fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) *(27)*. Several studies have shown that, in addition to lowering triglycerides, ω-3 fatty acids possess anti-inflammatory, antithrombotic, and antiarrhythmic properties *(28)*. In Lebanon, a study by Iskandar *(29)*, on adult subjects, showed that consumption of ω-3 fatty acid was inversely associated with many risk factors of non-communicable disease, including hypertension, low high-density lipoprotein (HDL) circulating levels, and hypertriglyceridemia. The study of Nasreddine et al. *(23)* also noted a decrease in daily olive oil consumption, which was estimated to be 5 g/d. This intake remains below the Food and Drug Administration daily recommended value of 23 g/d *(30)*. The protective effects of olive oil against coronary heart disease, various cancers and age-related cognitive decline are attributed to two fundamental components: monounsaturated fatty acids and antioxidant substances *(31)*. Olive oil exerts important effects on lipid profiles, including reduction of total and low-density lipoprotein (LDL) cholesterol, and increase of HDL cholesterol, and provides more resistance of LDL cholesterol to oxidation *(32)*. Moreover, despite the apparent decreased contribution of cereals (including bread) and the increased contribution of meat, fish, dairy, and eggs to daily energy and protein intake, a maintained consumption of roots, tubers, pulses, nuts, oilseeds, fruits, and vegetables provided significant sources of fiber, vitamins, minerals, and phytochemicals, known to be protective against many noncommunicable diseases.

Another peculiarity of the Lebanese diet is the high consumption of wild edible plants in poor communities of Lebanon *(33)*. A high percentage of individuals collect wild edible plants within selected villages, and consume them as foods. Akkoub (tumbleweed), babounij (camomile), farfahin (green purslane), hindbeh barrieh (chicory), khubbaizeh (mallow), qursaaneh (cryngo), sumac (sumach), and zaatar bari (wild thyme) are among the most consumed wild edible plants in Lebanon. These wild plants are thought to possess anti-cancerous and anti-inflammatory properties, to be rich in vitamins and minerals and to be beneficial for the heart, the skin and the gastrointestinal tract. More specifically, camomile was found to be rich in phenolics, associated with increased antibacterial activity and boosted immunity. It is rich in glycine, known to act as nerve relaxant. Chicory, rich in potassium, vitamin C, folate, and vitamin A, was reported to be beneficial for asthma, bruising, cancer, capillary fragility, cataracts, macular degeneration, multiple sclerosis, and stroke. As for mallow, being rich in mucilage,

flavonoids and anthocyanins, it was implicated in protection against irritation, mild and chronic inflammation, as well as autoimmune disorders and known to possess anticancer properties. Finally, wild thyme and its active extracts, thymol and carvacrol, were used as antimicrobial agents, as platelet aggregation inhibitors, as part of the therapy of chronic and acute bronchitis, as well as enhancers of the total antioxidant status.

In conclusion, the Lebanese diet is characterized by its favorable sensory properties and by its high popularity among the local population and neighboring countries. It has retained some of its healthy traditional Mediterranean characteristics, despite being influenced by the Western diet. Hence, current dietary habits are characterized by this duality *(34)*. It is still controversial to establish whether tradition or modernity is an exclusive prerogative of the Lebanese diet. In a globalized context, Lebanon is balancing between two reactions: the first is characterized by the acceptance and adaptation to modern norms and practices, whereas the second appears in the rejection and the refusal of this modernism *(19)*. Thus, and for the purpose of health preservation and maintenance of culture and tradition, efforts should be directed toward integrating healthy modern ingredients in the preparation of Lebanese foods. This would allow for the preservation of the healthy identity of the Lebanese Mediterranean diet, and protect its progress towards modernity.

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35 Fats and Fatty Acids in Nutrition of the Iranian People

Abolghassem Djazayery and Shima Jazayery

Abstract

Iran is undergoing an epidemiological and nutritional transition. Both undernutrition and nutrient deficiency diseases (e.g., anemia, iodine deficiency disorders, and some other mineral and vitamin deficiencies), as well as overnutrition in the of form of obesity, diabetes mellitus, cancers, and cardiovascular diseases, are present in sections of the population. The share of fat in the dietary energy intake is on the increase, proportions of MUFAs and UFAs are low, and that of saturated fatty acids is high. Furthermore, the average daily intake of ω-3 fatty acids and the ratio of ω-3-FA:ω-6-FA are very low, while that of trans-fatty acids is very high. Fats and fatty acids have received the attention of food and nutrition researchers. Research has been conducted in such areas as extraction, identification, and purification of ω-3 fatty acids from foods; effects of fats and ω-3 fatty acids in health and disease; finding ways to improve the technology of processing fats. Attempts to improve the quality of edible oils and dietary sources of ω-3 fatty acids have included production and marketing of ω-3 fatty acid-enriched eggs, experimental production of low trans-fatty acid oils and high ω-3 fatty acid-content oils with low perishability.

Key Words: Iran; fat intake; fatty acid intake; ω-3 fatty acids; trends of oil supply; dietary sources of ω-3 fatty acids; oil hydrogenation; fat contents of Iranian foods; cooking oil; dietary energy supply (DES).

1. INTRODUCTION

The Islamic Republic of Iran, with a surface area of 1648000 Km^2, is a predominantly agricultural country and quite diverse ecologically, geographically, climatologically, economically, sociologically, and culturally. As a result of effective population policies and programs, as well as socioeconomic changes over the last few decades, the annual population growth rate has had a downward trend, now being 1.07%. The total population, with a mean density of 40/Km, is 69,018,924 (46% under 18 yr of age). The infant mortality rate (IMR) is 42.8/1000 live births, life expectancy at birth is 70 years, and Human Development Index (HDI) 0.732. The economy is predominantly based on petroleum (the major source of foreign exchange) and the mean per capita Gross National Product (GNP) is USD 1734.00.

The main agricultural products are wheat, rice, pistachios, fruits, and vegetables. The most widely consumed food item, both in towns and villages, is cereals, providing about 2/3 of the total dietary energy supply, followed by dairy products, meat, oil + sugar, fruits, and vegetables in the food basket *(1)*.

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Table 1
Trends of the Average Shares of Macronutrients in the Iranian Dietary Energy Supply Between Mid-1980s and Early 2000s

	Average share (%)			
Period	*Protein*	*Carbohydrate*	*Fat*	*Ref.*
1984–1986	10.5	68.5	20.9	*1*
1996–1998	10.8	69.3	19.9	*1*
2000–2002	10.0	65.0	25.0	*4*

Table 2
Annual Trend of Edible Oil Supply in Iran Between 1991 and 2001 *(5)*

	Production		*Imports*		*Supply*		*Per capita*
	Total	*Per capita*	*Total*	*Per capita*	*Total*	*Per capita*	*supply*
Year	*(1000 tons)*	*(Kg)*	*(1000 tons)*	*(Kg)*	*(1000 tons)*	*(Kg)*	*(g/day)*
1991	138	2.5	550	12.3	677	21.1	31.6
2001	211	3.0	988	14.1	1134	17.0	47.0

Undesirable changes have occurred in the family food basket over the past two decades, such as increases in the mean amounts of oil and sugar, as well as increased popularity of fast foods *(2)*. These alterations in the types of dietary food items and food habits, plus reduced daily physical activity and overall changes in lifestyle, have resulted in increased risk factors for, and upward trends in, the prevalence of chronic nutritional diseases. Typical nutrition transition is occurring in Iran *(1–3)*. Both undernutrition and nutrient deficiencies (e.g., anemia, iodine deficiency disorders, and riboflavin deficiency), as well as overnutrition and diet-related diseases (e.g., obesity, cardiovascular diseases, diabetes mellitus, and cancers) are common problems in sections of the population. Population growth, socioeconomic changes, a rapid rate of urbanization, major changes in dietary habits, and low physical activity have all contributed to nutritional transition *(2,3)*.

2. DIETARY ENERGY SUPPLY, OIL AND FISH SUPPLIES, AND CONSUMPTION OF FOOD SOURCES OF ω-3 FATTY ACIDS

2.1. Dietary Energy Supply

The mean total daily dietary energy supply increased from 3052 kcals in 1991 to 3535 kcals in 2001, about 89% coming from plant sources *(1)*. It is worth noting that according to the available national data the average share of fat in the dietary energy supply is desirable (about 25%), within the current international recommendations of between 15 and 35%. According to Table 1, the shares of protein (about 10%) and carbohydrate (about 65%), also fall within the respective recommendations, between 10 and 12% and 55 and 75% and are very close to the global averages *(4)*. The important

Table 3
The Fatty Acid Composition (%) of Hydrogenated Vegetable Oils Produced in Iran in 2002 *(6)*

Fatty acid	*Mean ± SD*	*Range*	*Fatty acid*	*Mean ± SD*	*Range*
Myristic (14:0)	0.3 ± 0.1	0.0–0.4	Linoleic (18:*c*2)	6.5 ± 3.6	3.8–17.9
Palmitic (16:0)	11.5 ± 1.7	9.5–16.3	Linoleic (18:*t*2)	5.3 ± 1.5	1.7–7.6
Stearic (18:0)	9.0 ± 1.6	5.5–12.0	α-Linolenic (18:*c*3)	0.6 ± 0.41	0.6–1.4
Elaidic (18:t1)	32.3 ± 6.0	15.8–42.5	α-Linolenic (18:*t*3)	0.1 ± 0.05	0.0–0.0
Oleic (18:c1*)*	33.9 ± 4.9	21.4–41.4	Arachidic (20:0)	0.6 ± 0.13	0.0–0.7

thing is that the average share fat in the dietary energy supply has had an upward trend over the past 1.5 decades (Table 1).

2.2. Oil and Fish Supplies

2.2.1. Oil

Considerable changes have occurred in the supply, demand, and consumption of edible oil over the last 10 to 15 yr; both the total and per capita figures have been on the increase. Table 2 shows that both the domestic production and imports have had an upward trend (the amounts exported are small, equivalent to between 2 and 3% of the total supply). Consequently, the per capita daily supply increased nearly 50% between 1991 and 2001, from 31.6 g to 47.0 g.

The major oil seeds produced in Iran are soybeans and sunflower, together constituting 86% of the total national production. Other oil seeds cultivated are cottonseed, rapeseed, and safflower (*Carthamus tinctorius*). Less than 10% of the crude oil supply is provided by these sources, the rest being imported. Soybean oil is the major crude oil imported into the country. It is rich in ω-3 fatty acids, but the problem is that in the process of hydrogenation its trans-fatty acid content is increased considerably (*see* Section 6.2). According to a National Food and Nutrition Council Report in 2003 *(6)*, the fatty acid composition of the hydrogenated vegetable oils produced and marketed is that shown in Table 3. It is seen that the average ω-3, α-linolenic acid content is very low (0.60%); the range is quite wide, but even the upper end of the range (1.4%) is lower that the current recommendations. The mean ω-6, linoleic acid contents are, on the other hand, high. Another important fact that Table 3 shows is that the total proportion of the trans-fatty acids (elaidic, trans-α-linolenic, and trans-α-linoleic) of the hydrogenated oils is, on the average, 37.66%, which is very high and in reflected in the observed high intakes (*see* Section 3.2).

2.2.2. Fish

The supply of fish, the richest source of ω-3 fatty acids, and other aquatic food animals in Iran is low. The National Food Balance Sheets published by the Ministry of Agriculture revealed that between 1991 and 2001 the total annual fish production increased by 11.7% (from 358,000 to 400,000 tons), the per capita production staying virtually constant *(5)*. The mean per capita supply (equivalent to the production, because no fish is imported) is indeed very low, about 5 Kg/yr, as compared with the world average.

Table 4
The Average Weekly Consumption Frequencies of Selected Food Items in Iran From 1999 to 2000 *(7)*

	Weekly consumption frequency		
Food item	*Urban areas*	*Rural areas*	*Total*
Oil	9.2	9.6	9.4
Eggs	3.0	2.8	2.9
Fish	0.5	0.4	0.5

Table 5
Mean (±SE) Daily Intakes (g) of Selected Food Items in Iran From 2000 to 2002 *(4)*

	Area		
Food item	*Urban*	*Rural*	*Whole country*
Fish	5.0 ± 1.0	6.0 ± 1.0	6.0 ± 1.0
Other aquatic products	1.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.0
Eggs	2.0 ± 1.0	9.0 ± 1.0	21.0 ± 1.0
Total fats + oils	45.0 ± 1.0	46.0 ± 1.0	46.0 ± 1.0
Solid vegetable fat	36.0 ± 1.0	40.0 ± 1.0	37.0 ± 1.0
Liquid fat	3.0 ± 1.0	1.0 ± 1.0	3.0 ± 1.0
Butter	4.0 ± 1.0	2.0 ± 1.0	3.0 ± 1.0
Other	2.0 ± 1.0	3.0 ± 1.0	3.0 ± 1.0
Leafy vegetables	41.0 ± 1.0	24.0 ± 1.0	35.0 ± 1.0
Total vegetables	239.0 ± 1.9	210.0 ± 2.6	229.0 ± 1.5

2.3. Consumption of Food Sources of ω-3 Fatty Acids

The Second National Health and Disease Survey conducted from 1999 to 2000 showed that the consumption frequencies of selected food items, specifically oil, eggs, and fish, vary considerably *(7)*. The semiquantitative data (Table 4) obtained made it evident that the weekly consumption frequency of fish, the richest dietary source of ω-3 fatty acids, is the lowest (0.5 time/wk). The consumption of eggs, the other relatively rich source, is also low (2.9 times/wk); presently there are ω-3 fatty acid-enriched eggs on the market, but it is not known how much they contribute to the total intake of these fatty acids. On the other hand, the consumption frequency of oil, is quite high (9.4 times/wk).

More recent data on food consumption patterns have become available from another national survey conducted between 2000 and 2002 *(4)*. The findings show that the average per capita daily intakes of energy (2636 kcal) and protein (76 g) in both towns and villages are higher than the respective recommendations, in some provinces ranging from 35 to 50% higher. The proportions of individuals with an energy intake above 120% RDA ranges between 22% in Tehran Province and 70% in Ilam Province.

The daily intakes of fat and potential ω-3 fatty acid sources are depicted in Table 5. It is seen that the intakes of eggs, leafy vegetables, and particularly fish, are low. With regard to fat, the mean absolute amount of total fats + oils consumed daily is not high (Table 5), the major part is solid (i.e., hydrogenated, vegetable oil).

The share of fat in the dietary energy intake is 25%, and those of protein and carbohydrate, 10% and 65%, respectively–all acceptable according to current international recommendations.

3. FAT NUTRITION

3.1. Dietary Fat Intake

Jazayery et al. *(8)* reported that in Tehran, the capital and the largest city of the country, the proportion of dietary energy supplied by fat (26–28%) is somehow higher than the national average (24%), although it is still within the desirable range. These authors also showed that the proportion of dietary saturated fat is acceptable, 100% of the sample studied having an intake in line with the current international recommendations.

The results of other provincial and regional studies in the country generally are similar to those for Tehran. By far, the major part (90%) of dietary fat, whether produced domestically or imported, comes from plant sources. All these vegetable oils have a high ω-6 fatty acid and a low ω-3 fatty acid content; good sources of the latter (e.g., soybean oil), are not available or popular. Another problem is the high trans-fatty acid content of the hydrogenated vegetable oils commonly consumed (*see* Section 6.2). These findings would indicate that the problem of fat nutrition is more qualitative than quantitative.

The National Food and Nutrition Council (NFNC) analyzed the data obtained in the 1992–1995 national food consumption survey conducted by the Ministry of Agriculture and The National Nutrition and Food Technology Research Institute (NNFTRI) *(6)* and reported that the mean per capita total daily fat intake was 66.0 g (range of 22–85 g), equivalent to 22% of the daily energy intake. Analysis of the data also showed that the share of fat in the daily energy intake of 23.5% of the households studied was over 30%. About 85% of the total daily fat intake came from oils + fats (the major part in the form of hydrogenated vegetable oil), 1.5% from liquid vegetable oil, and most of the remaining 13.5% from animal fat *(6)*.

3.2. Fatty Acid Intake

The NFNC also states that saturated fatty acids constitute 13% of the total energy intake, somewhat higher than the current recommendations. Furthermore, the proportions of monounsaturated fatty acids (MUFAs) (7%) and polyunsaturated fatty acids (PUFAs) (1.5%), as well as the PUFA:MUFA ratio (0.21%) were all lower than the respective recommendations, whereas the intake of trans-fatty acids is much higher than the currently acceptable levels *(9)*. The mean daily ω-6 fatty acid intake as a proportion of the energy intake (1.5%) was satisfactory, but the ω-3 fatty acid (α-linoleic acid, EPA, and DHA) was only 0.06% *(9)*. These findings show the imbalance between the ω-3- and ω-6-fatty acids in the average Iranian diet and confirm findings reported by Khosravi et al. *(10)* who conducted a study in 1999 to determine the fat intake pattern in 10 provinces representing ecological, economic, social, and cultural diversity of the country. Based on the first national food consumption survey between 1992 and 1995 *(4)*, the average per capita energy and fat intakes were calculated, followed by the estimation of the quantities and types of fats and fatty acids and the cholesterol contents of the foods commonly consumed, using the national food composition tables and the available related information. The findings showed that the average share of fat in the dietary energy supply was between 16.5% (Kohkilouyeh-Boyrahmad Province villages in the

Table 6
Cholesterol and the Related Lipoprotein Profile in Tehran Province From 1999 to 2000 *(7)*

	Percentage of individuals with an undesirable profile		
Sex and age (yr)	*Total cholesterol (>200 mg/dL)*	*LDL-cholesterol (>135 mg/dL)*	*HDL-cholesterol (<35 mg/dL)*
Male			
40–69	50.1	37.6	32.7
70+	39.3	31.6	30.6
Female			
40–69	56.4	41.2	22.1
70+	51.9	35.6	16.7

south-west) and 29.8% (Tehran), falling within the current international recommendations. The saturated fatty acid (SFA), MUFA, and PUFA intakes in the provinces as a proportion of daily energy intakes ranged from 9.9 to14.8%, 6.1 to 9.1%, and 1.7, to 3.4%, respectively, the latter two well below recommendations. The PUFA/SAF ratio, too, was much below the accepted level of 1, ranging from 0.14 to 0.22. The findings also showed that while sufficient amounts of the ω-6 linoleic acid were consumed in all but 3 of the smaller provinces (1% of the daily energy intake), the picture of ω-3 fatty acids was not satisfactory at all. In 9 of the 10 provinces the EPA + DHA intake was only 0.02 to 0.18/d, well below current recommendations of 0.3-0.4g/d. Only in Hormozgan, situated along the Persian Gulf in the south, was the intake at a desirable level (i.e., 0.36g/d).

3.3. Blood Cholesterol Profile

The plasma cholesterol-carrying lipoprotein profile in Tehran Province (Table 6), where an ethnically, economically and culturally diverse population equivalent to nearly one-sixth of the total Iranian population lives, can be said to at least partially reflect these facts about the quality of the dietary fat.

4. EXTRACTION AND IDENTIFICATION OF THE ω-3 FATTY ACIDS

Attempts have been made to find new potential food sources of ω-3 fatty acids and extract these fatty acid from various plant and animal sources, identify and purify them for practical applications.

4.1. Animal Sources

Considering the beneficial nutritional effects of fish oil, a group of food scientists extracted oil from various marine fish and crude fish oil contained in the fish powder production plant wastes *(11)*. The physical and chemical characteristics of the extracted oils, as well as their shelf-life and ω-3 (and other) fatty acids contents, were studied and found to be desirable. The same workers have also suggested shark liver oil to be used as a potential source of ω-3 fatty acids *(12)*. The suggestion was based on their findings that showed the oil could be extracted from shark liver, purified and deodorized to obtain an oil with desirable organoleptic properties and a reasonable shelf-life.

In another study, fat was extracted from the sturgeon processing plant wastes; this fish is obtained from the Caspian Sea in the North of Iran and exported. Analysis of the

abdominal fat of this kind of fish showed that its content of ω-3 fatty acids was about 10% and that it would be possible, using appropriate methods, to get ω-3 fatty acids with 90% purity *(13)*. The authors recommended to make use of this potential source of ω-3 fatty acids at an industrial scale.

By far the two major sources of marine fish in the Iranian diet are the Caspian Sea in the north and the Persian Gulf in the south. Most fish found on the market come from one or the other of these two sources. Some investigators have compared the fat contents and the fatty acid compositions of the southern and northern fish *(14)*. Three strains from either region were included in the study, 6 males and 6 females from each strain. Fat was extracted, homogenized, and analyzed for fatty acids using gas chromatography. The findings showed that the most fatty strains were tigertooth croaker (*Otolithes rubber;* local Iranian name *Shoorideh*) from the south (4.6% fat) and kutum (*Rutilus frisii*; local Iranian name *white fish*) from the north (3.9% fat). The average ω-3 fatty acid contents of the northern and southern fish were 28.4 ± 8.1 and 22.9 ± 4.8 g/100 g fat, respectively, the difference being statistically significant ($p < 0.05$). The highest contents (per g fat) were found in carp from the south (31.3 ± 5 g/100 g) and kutum from the north (32.7 ± 9.1 g/100 g). Comparing the fatty acid contents of the muscles revealed tigertooth croaker and kutum to have the highest contents, 1070.5 ± 250.7 and 1271.6 ± 261.0 mg/100 g, respectively.

The data also showed that the average ω-6 fatty acid content of the southern fish was 12.7 ± 4.6, whereas that of the northern fish was 9.1 ± 3.0, mg/100 g fat. Further analysis of the data indicated the ratio ω-3 fatty acid:ω-6 fatty acid to be 5.2 in white fish and 3.0 in tigertooth. The authors concluded that these 2 types of fish were the best sources of ω-3 fatty acid with a reasonable ω-6 fatty acid:ω-3 fatty acid ratio.

4.2. Plant Sources

Several workers have investigated the potential plant sources of fatty acids. For example, the fat and fatty acid contents of several plant seeds (and some other plant parts) were determined in Ahvaz, south-west of Iran *(15)*. The seeds are not a main dietary ingredient, but are often used more or less as snacks ("nuts") in that part of the country. Table 7 summarizes the findings.

In another study, the fatty acids of a large number of fruit stones were determined *(16)*. The results of the study are shown in Table 8. What prompted the investigators to undertake this effort was that fruits are, in general, popular in Iran; the types grown depend on the region in the country. As mentioned above, the fruit stones, too, are mainly used as snacks. Consequently, if they are found to have considerable amounts of ω-3 fatty acids, their consumption could be recommended both as a food item and for pharmaceutical purposes—considering that the average, typical diet is poor in ω-3 fatty acids. As Table 8 shows, the fat contents and fatty acid compositions of different fruit stones vary considerably. The fatty acid composition of the seed oil of *Badamak* (*Amygdalus scoparia*), a kind of mountain almond, is very close to that of pistachio nuts. It is also seen that the oleic acid content of several of the oils listed is quite high, and some of them are rich in linoleic acid.

More recently, another report appeared in the literature on the fatty acid contents of several Iranian nut oils *(17)*. As can be seen from Table 9, all, except walnut oil, are very rich sources of oleic acid, whereas the one most similar to olive oil as regards total fatty acid composition is hazelnut oil. Table 9 also shows that the only oil that contains relatively significant amounts of α-linolenic acid is walnut oil; it is even richer than soybean oil. The

Table 7
Total Fat and Fatty Acid Contents of Some Plants in Iran *(15)*

Plant type (part)	*Total fat (% on dry weight basis)*	*Fatty acids (% total fat)*			
		Saturated		*Unsaturated**	
		Palmitic	*Stearic*	*Oleic*	*Linoleic*
Watermelon, sugar baby variety (seed)	40	12	6	10	62
Watermelon, Charleston variety (seed)	61	11	5	15	66
Pistachio (nuts), *Pistacia vera*	38	10	–	69	28
Badamak (seeds), Amygdalus scorpia	47	9	1	69	21
Hybrid corn double cross (710)-Zea mays	7.5	12	–	21	55
Beneh (fruit), *Pistacia mutica*	58	12	1	57	19
Kolkhong (fruit), *Pistacia khinjuk*	56	20	2	59	16

*α-Linolenic acid was found in trace amounts in all these plants.

ratio ω-6 fatty acid: ω-3 fatty acid in walnut oil is 4.5, which is much more desirable than that in pistachio oil—38.6. Neither of the two nor the other nut oils shown in Table 9, however, are popular in the Iranian diet, but the nuts themselves are common.

Finally, it has been shown that common purslane (*Portulaca oleraceae*), a green leafy vegetable which grows in some parts of Iran, contains reasonable amounts of α-linolenic acid (24% of the total fat), although it has very low contents of the longer-chain EPA and DHA *(18)*. The linoleic content is high, accounting for more than 33% of the total fat.

5. RESEARCH ON THE EFFECTS OF ω-3 FATTY ACIDS AND THEIR FOOD SOURCES IN HEALTH AND DISEASE

5.1. Animal Work

A few investigators have compared nonhydrogenated liquid oil with hydrogenated vegetable oil with regard to their effects on the blood lipid profile in experimental animals. A group of workers fed different age/sex rats with either liquid or hydrogenated corn oil for a total of 6 mo *(19)*. Their findings showed that, as compared with the initial values, there were statistically significant decreases in the serum triglycerides (15%) and low-density lipoprotein (LDL)-cholesterol (68%), as well as significant increases in high-density lipoprotein (HDL)-cholesterol (32%) in the liquid oil group at the end of the period. The hydrogenated oil produced different effects: a significant increase (37%) in the serum triglyceride level after 4 mo, with no changes in LDL- or HDL-cholesterol. Neither of the 2 oils brought about a change in the serum total cholesterol level even after 6 mo–a finding that need not cause concern, bearing in mind that what matters most is desirable changes in LDL- and HDL-cholesterol, rather than total cholesterol.

Table 8
Moisture, Fat, and Fatty Acid Contents of Iranian Fruit Stones *(16)*

			Fatty acids (% total fat)					
			Saturated			*Unsaturated*		
Fruit type	*% Moisture*	*% Fat*	*Myristic*	*Palmitic*	*Stearic*	*Oleic*	*Linoleic*	*α-Linolenic*
Apple-1	19.15	30.43	0.05	8.04	1.80	38.20	49.92	1.50
Apple-2	28.23	27.31	0.04	8.88	1.09	32.35	56.96	0.68
Apple-3	17.14	30.09	Traces	8.90	1.64	34.34	52.37	0.76
Pear-1	15.10	34.13	Traces	6.93	3.28	49.50	38.83	1.46
Pear-2	20.90	32.83	Traces	9.27	1.63	33.29	53.76	2.05
Cherries	20.40	39.82	Traces	8.47	2.63	52.78	35.16	0.68
Sour cherries	23.90	22.74	0.06	39.70	1.81	40.62	49.10	0.37
Grapes	10.47	25.16	0.05	9.61	3.31	21.19	65.52	0.32
Grapefruit	25.80	53.62	0.07	29.49	2.91	23.06	38.25	6.15
Orange-1	28.80	43.50	0.03	5.87	4.42	25.88	39.20	4.50
Orange-2	39.78	36.77	0.10	29.20	3.33	29.80	34.31	3.20
Sour orange	30.92	52.34	0.04	26.47	4.66	27.44	32.94	8.57
Sour lemon	11.51	54.86	0.15	30.34	3.43	24.92	33.49	7.56
Sweet lemon	26.19	47.12	0.08	28.03	1.83	27.80	37.36	4.81
Quince	4.49	34.48	0.03	6.05	1.01	45.15	46.94	0.56
Tangerine	14.80	46.01	0.03	25.97	3.86	25.42	39.55	5.07

Table 9
Fatty Acid Contents of Some Iranian Nut Oils Compared With Olive Oil *(17)*

		Fatty acids (% of oil)					
Type	*% Fat*	*Plamitic*	*Palmitoleic*	*Stearic*	*Oleic*	*Linoleic*	*α-Linolenic*
Pistachio	40.8	10.6	0.7	0.7	60.3	27.0	0.7
Hazelnut	59.1	5.2	Traces	2.0	83.5	9.3	Traces
Walnut	54.1	5.7	Traces	2.2	20.9	57.9	13.3
Almond	50.1	6.9	0.4	1.4	65.9	25.5	Traces
Olive oil		*11.0*	*0.6*	*2.6*	*78.6*	*7.2*	*Traces*

Another group of investigators (20) compared the effect of the rabbit serum lipoproteins of diets in which the source of fat (8%) was either liquid olive oil, hydrogenated corn oil, or a mixture of the two. All 3 diets caused significant increases in the serum triglyceride, and LDL-cholesterol levels after 12 wk, the increases being highest in the hydrogenated oil group. Comparison between the mixed oil and the hydrogenated oil group revealed a significantly lower level of the serum triglyceride in the former. There was no histological evidence of the existence of any differences among the 3 groups

with regard to the aortic sclerotic lesions after 12 wk. The authors concluded that the liquid olive oil with a high monounsaturated fatty acid content can counteract the undesirable effects of hydrogenated oil on the serum lipid levels.

Comparisons have also been made between hydrogenated vegetable oil and animal fat. Two groups of male albino 1-mo-old rabbits were put on a diet whose fat source was either hydrogenated cottonseed oil or animal fat for a period of 3 mo. Initial and final measurements of serum lipoproteins showed that, as compared with the control group (fed a stock diet), both oils brought about statistically significant increases in the serum total cholesterol, VLDL-cholesterol, and triglycerides. The data also showed that although there were significant increases in LDL- and HDL-cholesterol resulting from the hydrogenated oil, there were only small, statistically nonsignificant elevations as a result of animal fat feeding *(21)*. Based on their findings, the investigators hypothesized that animal fat may decrease cardiovascular disease risk to some extent, because it does not increase the serum LDL-cholesterol.

Some workers have investigated the effect of ω-3 fatty acids in disease conditions in animals. In a study aiming at finding the effect of fish oil on nerve conduction in diabetic rats, two groups of rats were made diabetic with alloxan and put on an ordinary stock diet *(22)*. After a lapse of 10 d, one group (experimental) was fed, in addition to the diet, fish oil in an amount equivalent to 10% (by weight) of the diet by tube-feeding for another 10 d; the other group with no change in their diet, served as the control. Measurement of the speed of nerve conduction showed a statistically significant 8.5% increase in the speed in the experimental group, as compared with the control group ($p < 0.05$). Probable reasons for such a change would be increased red blood cell plasticity, lower blood viscosity, reduced platelet aggregation, and changes in the endoneural blood flow in the sciatic nerve. There is evidence that ω-3 fatty acids can exert some of these effects *(23,24)*.

Other workers have compared the effects of fish and corn oils, tube-fed for 4 wk, on wound healing in diabetic mice. It seems that fish oil can play a significant role in enhancing speed of wound healing in mice with alloxan-diabetes *(25)*. Findings of the same study, in which the change in the surface area of an induced wound was used as a criterion for the speed of wound healing, indicated that corn oil actually *delayed* healing; the differences were statistically significant in all cases. These results have been confirmed by other investigators *(26)*, who also reported that a possible explanation for the observed enhancive role of fish oil in wound healing is increased blood flow and decreased inflammation in the wound area due to the high ω-3 fatty acid content of fish oil; there is evidence on the anti-inflammatory effects of ω-3 fatty acids *(27)*.

5.2. Human Studies

Not many clinical trials have been conducted in Iran to determine the effects of feeding ω-3 fatty acid food sources or supplements. Basically, that is to say, physiologically, there is no reason why the effects should necessarily be different in individuals in different countries or regions, because basic physiological and metabolic processes and mechanisms are, on the whole, the same in all human beings. However, depending on the baseline conditions of an individual (e.g., his/her initial nutritional, fat, and ω-3 fatty acid status), increased intake may produce effects to various degrees. For this reason, it is reasonable and justified to conduct clinical trials in various populations.

Taleban et al. *(28)* conducted a study on Tehrani healthy male, nonsmoking college students about 23 yr old, with a body weight and body mass index of 65 kg and 21.5,

respectively, and with no family history of heart attack below 50 yr of age. They were fed marine fish as part of their diet (evening meal) for 5 wk; initial and final blood lipid profiles were compared. The fish, coming from either the Caspian Sea in the north or the Persian Gulf in the south of Iran, was supplied daily as 2300-mg portions fried in hydrogenated vegetable oil, the most popular frying oil in the country. The Caspian Sea fish caused a statistically significant reduction in the TG level (from 146.1 ± 32.2 to 108.0 ± 20.6 mg/100 dL; $p < 0.01$) and in the VLDL-cholesterol level (from 29.7 ± 5.2 to 22.6 ± 4.1 mg/100 dL; $p < 0.05$), as well as an increase in the HDL-cholesterol concentration (from 43.5 ± 5.4 to 51.8 ± 8.0 mg/dL; $p < 0.05$). The corresponding decreases and increases resulting from the Persian Gulf fish were statistically nonsignificant. Further analysis of the data showed changes neither in total cholesterol, LDL-cholesterol, or blood pressure, nor in body weight, daily energy intake (about 10.9 Mj), or macronutrient shares in dietary energy (63%, 27%, and 10%, from carbohydrates, fat, and protein, respectively), in either of the 2 groups.

The second clinical trial in healthy subjects aimed at studying the effects of dietary ω-3 fatty acid supplementation of postmenopausal women on some of the blood-related cardiovascular risk factors *(29)*. The experimental group was fed 31-g ω-3 fatty acid capsules/d for 28 d: the controls were fed a placebo. The findings showed statistically significant decreases in the serum TG level, as compared to the initial value, as well as to the final control value. Similarly, the ratio TG/HDL-cholesterol decreased significantly in the experimental group, the final value being also significantly lower than the respective control value. No changes were observed in other lipid fractions or in apo-B.

A more recent report describes the anti-inflammatory and immunoregulatory effects of ω-3 fatty acid-enriched eggs in healthy adults. Twenty-one normocholesterolemic, normoglycemic, and normotriglyceridemic college students were fed 2 ω-3 fatty acid-enriched eggs/d for 8 wk; the control group took 2 ordinary eggs/d. The results showed reductions in the fasting plasma insulin level from 0.32 ± 0.03 to 0.23 ± 0.02 ng/ml ($p < 0.02$) and in hsCRP from 0.36 ± 0.02 to 0.31 ± 0.01 mg/dL ($p < 0.03$) in the experimental group. No other statistically significant changes occurred *(30)*. These findings are an indication of the beneficial effects of ω-3 fatty acid-enriched eggs with regard to reducing the risk/progression of atherosclerosis (an inflammatory disease) and diabetes.

Some investigators have become interested in the potential effects of ω-3 fatty acids in disease conditions. A group of investigators *(31)* fed, in a double-blind-placebo-controlled trial, 4 matched groups of hyperlipidemic (TG > 200, or total cholesterol > 240, mg/dL) hemodialysis patients a daily supplement of either ω-3 fatty acids (2 g), the antioxidant vitamin E (300 mg), a combination of the 2, or a placebo (control group). The blood levels of lipid fractions, anthropometric measurements, and dietary intakes (using the 24-h dietary recall technique) of the four groups were compared at weekly intervals. Only after 5 wk did a statistically significant difference appeared between the control and the 3 experimental groups—a reduction of 24.1% as compared with the control's TG level ($p < 0.02$). Supplementation for 10 wk resulted in a significant decrease (18.6%; $p < 0.05$) in the plasma LDL-cholesterol of the ω-3 fatty acid group. In addition, two significant changes were observed in the vitamin E group: (i) a decrease in the LDL-cholesterol/HDL-cholesterol ratio ($p < 0.05$) and (ii) an increase in the plasma vitamin E level ($p < 0.05$). There were either no or small, statistically non-significant, changes in the other variables tested (body weight, BMI, energy and nutrient intakes) in any of the groups.

The comparative effects of supplementation with ω-3 fatty acids and ascorbic acid (another antioxidant vitamin) on plasma lipoproteins, ApoA-1, ApoB, and malondialdehyde (MDA) have also been studied in hyperlipidemic subjects *(32)*. A group of 68 hyperlipidemic patients (both total cholesterol and TG > 200 mg/dL) were randomly assigned to either one or another of 3 daily supplements: (i) 500 mg ascorbic acid, (ii) 1 g ω-3 fatty acids (SuperEPA), or (iii) a combination of both; the fourth group receiving a placebo served as control. Fasting blood samples were collected at the beginning and after a 10-wk period and analyzed. Comparison between the food consumption pattern and socioeconomic and anthropometric parameters showed no significant changes during the period. The findings showed that the ω-3 fatty acid supplementation had brought about significant decreases in the TG level (from 304.6 ± 88.5 to 211.7 ± 78.4 mg/dL; $p = 0.02$), the TG/HDL-cholesterol ratio (from 8.1 ± 2.9 to 6.1 ± 2.9; $p = 0.04$), and the ApoB concentration (from 148.2 ± 26.8 to 134.6 ± 27.2 mg/dL; $p = 0.05$). No changes occurred in LDL-cholesterol, ApoB, or MDA, and the decrease in the total cholesterol (from 243.4 ± 3.6 to 229.0 ± 48.1 mg/dL) did not reach statistical significance. The authors observed no beneficial effects on the blood lipid profile of the subjects due to simultaneous administration of ω-3 fatty acids and ascorbic acid, although they found significant decreases in ApoB and MDA. Supplementation with ascorbic acid alone caused significant decreases in total cholesterol, ApoB, and MDA.

Another report describes the results of a study aiming at finding how the blood lipid profile and anthropometric measurements of hypercholesterolemic women were influenced by walnuts, a relatively popular nut rich in ω-3 fatty acids *(33)*. The diet of a group of mildly hypercholesterolemic postmenopausal women was supplemented with 27 to 35 g walnuts/d, replacing 1/3 of the total fat intake, for 4 wk. Food intake measurement, using the 24-h dietary recall technique, showed an increase in the daily α-linolenic intake from 0.48 ± 0.28 g in the week 1 to 2.6 ± 0.04 g in week 4. Several statistically significant desirable changes occurred by the end of the period: decreases in total plasma cholesterol (15.0%; $p < 0.001$), LDL-cholesterol (25.7%; $p < 0.01$), and the ratio LDL-cholesterol/HDL-cholesterol (29.1%; $p < 0.05$); the changes in TG, HDL-cholesterol, and the ratio total cholesterol/HDL-cholesterol were nonsignificant. Zibaeenezhad et al. *(34),* however, found a statistically significant reduction (19–30%) only in the plasma TG level of a group of adult hyperlipidemic subjects whose diets had been supplemented for 8 wk with 3 g walnut oil/day.

6. COOKING OIL

6.1. Cooking Oil in the Food Industry

Studies have been conducted on the quality of frying oils used in the industrial process of potato chips (potato crisps) production, because this food item is very popular, particularly among children. Afshinfar and Zandi *(35)* reported that the repeated use of the vegetable oils in the process causes undesirable changes in their fatty acid composition. Their data showed a reduction of up to 36% in the α-linolenic acid content of the frying oil as a result of repeated heating, whereas the decrease in the linoleic acid level was only 2.7% at the maximum; this means that the ratio of the former to the latter decreased considerably. The content of palmitic acid, the major saturated fatty acid, increased by 5.0% under conditions of the experiment.

In another study, the same group of workers investigated the effect of heating on the free fatty acid content and chemical characteristics of a mixture of soybean and sunflower oils with added antioxidants used in a potato-chips manufacturing plant *(36)*. Such a mixture would be repeatedly used for 20 d, about 4 hours/d, each day some fresh oil, equivalent to 20%, being added to it; the frying temperature was 133 ± 7ºC. The findings revealed that the initial free fatty acid content of 0.02% had increased 10-fold to 0.20% at the end of the 20-d period. Under the same processing conditions there were also decreases in the iodine number from 123 to 109 and in the smoking point from 225°C to 106°C. The authors concluded that heating the oil mixture was done for too long a period, leading to undesirable changes in its characteristics.

6.2. Hydrogenation and the Fatty Acid Composition of Cooking Oils

Hydrogenation of vegetable oil can inadvertently change the *cis* configuration of some of the double bonds in the fatty acid molecules into the trans configuration, which will increase the atherogenecity of the oil. This has received the attention of some investigators, prompting them to conduct research in an attempt to find ways to minimize it. Presently, a considerable part of the cooking oil marketed in Iran is semi-solid, hydrogenated vegetable oil. Investigations have been conducted into the effect of hydrogenation on the oil fatty acid composition under the processing conditions prevailing in Iran, as well as finding alternative methods for it, aiming at avoiding, or at least reducing, its undesirable effects. A study in 1997 revealed the following: (i) the major fatty acids in the nonhydrogenated liquid oils are linoleic and α-linolenic acids, the levels of both of which decrease significantly ($p < 0.05$) as a result of hydrogenation; (ii) in the hydrogenated oils the major fatty acids are elaidic acid—the trans isomer of oleic acid; and (iii) the ratio of MUFA to PUFA, which is 11.5 in the liquid oil, decreases to 1.3 in the hydrogenated product *(37)*. The authors recommended that efforts should be made in the oil processing industries to use methods which would result in less undesirable changes in cooking oils, particularly as regards the *trans* configuration production.

6.3. Attempts to Find Ways to Minimize Undesirable Effects of Oil Hydrogenation

It was probably that recommendation that prompted other workers to conduct research to find how interesterification of oils could help produce an oil with more desirable characteristics as compared with the hydrogenated oils. Inter-esterification is, in effect, an alternative method of "relative hydrogenation" of an oil, presently being used at the industrial scale in some countries. It involves essentially displacement of acyl groups within the triglyceride molecule, resulting in an oil in which the chemical characteristics of the constituent fatty acids in general, and the degree of unsaturation of PUFAs in particular, change little, and no geometrical or positional isomerization of the fatty acids occurs *(38)*.

In 2003, a group of workers *(39)* conducted a study on the possibility of interesterification in a mixture containing equal amounts of sunflower, soybean, cottonseed, and hydrogenated soybean (soy flakes) oils, using a 1,2,3-random distribution method with sodium-methoxide as catalyst. Inter-esterification for 30 min at 110°C brought about desirable improvements in the physical and chemical properties of the oil mixture and a reduction of the solid fat content from 17–30% to 1–14%, depending on the storage

temperature (10–40°C), as well as a significant decrease in the slip melting point from 54 to 35°C, important both nutritionally and technologically. The findings also showed that no undesirable changes in the chemical structure of fatty acids had occurred. In the final mixture there were only trace amounts of trans-fatty acids (0.21% elaidic acid from soy flake). This is extremely significant, because presently the proportion of trans-fatty acids in the widely consumed hydrogenated oils in Iran is of the order of 20 to 30%. Further analysis of the data showed that the unsaturated fatty acid contents of the mixture had, on the other hand, increased.

7. SUMMARY AND RECOMMENDATIONS

Iran is undergoing an epidemiological and nutritional transition. The average energy and protein intakes are higher than current international recommendations. However, both undernutrition and nutrient deficiency diseases (e.g., anemia, iodine deficiency disorders, and some other mineral and vitamin deficiencies) as well as overnutrition and conditions resulting from an imbalanced diet, in the of form of obesity, diabetes mellitus, cancers, and cardiovascular diseases, are present in sections of the population. The share of fat in the dietary energy intake is on the increase, proportions of MUFAs and PUFAs are low, and that of saturated fatty acids is high. Furthermore, the average daily intake of ω-3 fatty acids and the ratio of ω-3-FA:ω-6-FA are very low, while that of trans-fatty acids is very high. The available information also shows that in 1/3 to 1/2 of the population the blood cholesterol profile is undesirable.

For reasons mentioned above, fats and fatty acids have received the attention of food and nutrition researchers and, though to a lesser extent, food producers and processors. Research has been conducted mainly in the form of master's, doctoral (PhD), MD, and Pharm D theses. The areas covered have been extraction, identification, and purification of ω-3 fatty acids from dietary sources of fat; effects of fats and ω-3 fatty acids on man and experimental animals in health and disease; finding ways to improve the technology of processing fats, particularly ways to reduce inadvertent production of trans-fatty acids and improve the quality of certain seed oils produced.

Attempts to improve the quality of edible oils and dietary sources of ω-3 fatty acids have included production and marketing of ω-3 fatty acid-enriched eggs, experimental production of low trans-fatty acid oils and high ω-3 fatty acid-content oils with low perishability. Also, some experts are trying to prepare labeling regulations for mentioning fatty acid composition of foodstuffs (e.g., eggs, on the labels).

Another activity has been in the area of public education on the nutritional value of eggs (and to a lesser extent, fish and other marine foods). The private sector, nongovernmental organizations, the relevant government departments (e.g., Ministry of Agriculture, Ministry of Health and Medical Education) and egg producers have all been involved. A National Committee for Correcting Egg Consumption Pattern has been formed which has organized so far 2 1-d seminars with the collaboration of the concerned ministries, NGOs and the private sector.

It is recommended that the following activities be initiated/expanded:

- Identifying new sources of edible oil-seeds.
- Exploiting biotechnological/genetic methods to produce seed oils with a more desirable fatty acid composition requiring minimum industrial processing.

- Promoting consumption of existing rich sources of ω-3 fatty acids (e.g., walnuts) through public education.
- With regard to fish and fish oil: Increasing domestic production of fish and fish oil; improving fish oil processing methods aiming at obtaining a more desirable fatty acid pattern; using fish oil in new product formulations.
- Using oil processing methods that will result in production of less trans-fatty acids.

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36 Southeast Asian Diets and Health Promotion

Buncha Ooraikul, Anchalee Sirichote, and Sunisa Siripongvutikorn

Abstract

Southeast Asia, the food bowl of Asia, covers 11 countries (i.e. Brunei, Cambodia, East Timor, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Thailand, and Vietnam). Their diet consists principally of rice or noodles as the staple food, fish as the main source of protein, fruits and vegetables, and herbs and spices. Their cooking style is strongly influenced by the Chinese, Indian, and Malay culinary traditions. Their food ranges from the rich and spicy dishes of the Muslims and the Indians in Brunei, East Timor, Indonesia, Malaysia, and Singapore—the milder and less spicy local cuisines of the rest of the countries—to the relatively plain Chinese dishes among the Chinese communities in most cities. Perhaps the most distinguishing characteristics of Southeast Asian diet are the abundance of seafood and fruits and vegetables, and the liberal use of herbs and spices. Fish is considered the healthiest source of protein, made even more nutritious when it contains ω–3 and ω–6 fatty acids. Fruits and vegetables are excellent sources of vitamins, minerals, dietary fiber, and the nutritionally extremely important phytochemicals. Spices enhance the flavor and taste of the food, but also add nutritional and medicinal value to the dishes because of their biologically active phytochemicals. Generally, food of Southeast Asia is considered healthy as evidenced largely by the much lower incidence of acute obesity and cardiovascular disease as compared with the West. Unfortunately, with the rapid development and industrialization in these countries, the food trend is moving away from the traditional rice and food with lots of fruits and vegetables to the Western-style fast food and protein-rich diets, with extremely damaging consequence, especially in big cities.

Key Words: Southeast Asia; diets; food; health promotion; native dishes; fish diet; rice; spices; fruits and vegetables.

1. INTRODUCTION

Southeast (SE) Asia is made up of 11 countries (i.e., Brunei Darussalam, Cambodia, East Timor, Indonesia, Laos, Malaysia, Myanmar (Burma), the Philippines, Singapore, Thailand, and Vietnam). It has traditionally been considered the food bowl of Asia, being blessed with fertile soil, warm and humid climate, and monsoon rains, ideal for any crops and animals of tropical and subtropical varieties. It is a melting pot of racial and ethnic groups, ranging from the native Orang Asli and Sakai in Malaysia, the various Hill-tribes in Thailand, Myanmar and Laos, to the ethnic Malays, Chinese and Indians, and, more recently, the Europeans and the Americans, and, of course, the Eurasians. It is not surprising, therefore, that almost a whole range of social, religious,

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architectural, and food cultures are apparent everywhere in this region. Obviously, one cannot write about the diets and how they affect the health of the people of Southeast Asia without considering their foods and eating habits and how these are affected by their social, religious and cultural practices.

The distinguishing characteristics of the Southeast (SE) Asian diet may be summed up into five groups of food—rice, noodles, fish, fruits and vegetables, and spices. The following is a brief review of these food groups, how they feature in the SE Asian diets and their major nutritional and medicinal properties.

1.1. Rice

Rice is the cereal grown and consumed as the main staple throughout the region. In fact, it is the staple of more than half of the world population. There are two main varieties of rice consumed in SE Asia—regular long grain rice and glutinous or sticky rice. The former is, by far, the most commonly consumed variety, whereas glutinous rice is a popular staple among mainly the Laotians and the Northeastern and Northern ethnic Thais. Thailand is the biggest rice producer in the region, and the number one world rice exporter, but Vietnam, Myanmar, the Philippines, Cambodia, and Laos are also major producers and exporters. There are many varieties of rice grown in the region, but perhaps the best known and most popular for its eating quality is the fragrant "jasmine rice" grown in Thailand.

Rice grains are encased in the husk, which has to be removed in the first step of the milling process to produce "unpolished" or brown rice. The unpolished rice is the edible part of the grain consisting of endosperm, germ, and aleurone layer (bran). Endosperm, the main part of the grain, is packed with starch granules, which give characteristic eating quality to rice when cooked in water. Rice starch granule is unique in that it is one of the smallest when compared with starch granules from other plant sources. Its functional property as a food ingredient has been of recent interest to the food industry. Key applications for the ingredient with a tiny granule size, neutral taste, and soft mouthfeel include baby food, extruded products, soups, fat replacer, dressings and meat preparations *(1–3)*. Rice endosperm also contains a small amount of protein and, in pigmented varieties, some flavonoids.

The germ or embryo is a small organ attached to the base of the grain, from which the root and leaf are formed when it sprouts. It is high in lipids, nitrogenous compounds, including some vitamins e.g. tochopherols, and minerals.

The aleurone layer or bran forms the coating of the grain. It consists principally of cellulose, hemicellulose, some lignins, lipids and minerals. Rice bran oil containing γ-oryzanol, a mixture of ferulic acid ester sterol and triterpene alcohol with antioxidant properties *(4,5)* and dietary fiber are considered to be of high nutritive value. The vitamin-E group antioxidants, consisting of tocopherol, oryzanol, and tocotrienol oryzanol which occur naturally in rice bran oil are reported to be the key elements responsible for the reduction of the harmful cholesterol (low-density lipoprotein [LDL]) without reducing the good cholesterol (high-density lipoprotein [HDL]). Tocotrienol is considered the most powerful vitamin E existing in nature and is said to have an anticancer effect *(6)*. Rice germ and bran contain estrogens, which include specific estrogens such as isoflavones and coumestans found to be effective against various forms of cancer *(7,8)*. Unfortunately, when rice is milled to produce "polished" or white rice

most of the germ and bran is removed and, with it, most of the nutrients. Promotion is afoot, especially in Thailand, to persuade people to consume more unpolished rice, which is much more nutritious than white rice. Unfortunately, tradition and preference die hard, and the promotion has not met with overwhelming success.

There have been numerous research efforts to improve nutritional value of rice and to develop new uses for rice grain and/or its various components. It has been shown that germination of rice grain, whole or unpolished, results in multifold increase of vitamins, antioxidants and many other nutritionally important compounds such as γ–aminobutyric acid (GABA), enzymes and hormones *(9,10)*. Germinated unpolished rice also has superior cooking and eating qualities as compared with its un-germinated counterpart, which is normally hard and has unappealing flavor and taste after cooking. Young rice shoots have been harvested and processed into a functional drink or dry functional ingredient containing high amounts of antioxidants, vitamins (e.g., ascorbic and folic acids), and minerals (e.g., ionizable iron) *(11)*, as well as monosaccharides (e.g,. fructose and glucose) *(12)*. Rice grains have been fractionated into starch, protein, lipids, and fiber, having functional and nutritional properties desirable as ingredients in many regular and functional food products *(2,3)*.

1.2. Noodles

Noodles are probably the second largest staple food in the region. The majority of noodles consumed in SE Asia are made from rice flour, though wheat-based noodles are also quite popular, especially with the more recent advent of "instant noodles" and the economic necessity caused by the 1997 economic crisis. Noodle, of course, is the product of Chinese influence and its consumption as a staple food is almost as high as rice in Chinese communities. A greater variety of noodles are now available on the market, mainly to serve varying tastes and provide higher nutritional value. Egg noodles, vegetable noodles, noodles fortified with high dietary fiber, minerals or vitamins, and silver noodles made from mung bean starch are just some examples. They may come in flat strips, or thick or thin vermicelli styles, or even rectangular shapes, and their methods of preparation are just as numerous. They may be served fried in oil, with meat, vegetables, sauces, and spices, or they may be cooked and served with gravy of meat, vegetables, sauces and spices on top, or they may be served in various plain or spicy soups, again with meat, vegetables, sauces and spices. They may be consumed as the main meal, normally for lunch, or as snack at anytime of the day. Their popularity spans across racial, cultural and even religious groups.

1.3. Fish

One common characteristic of people in SE Asian countries is that their main source of protein, or about 55% of animal protein intake *(13)*, is fish or other aquatic animals, both from fresh and saltwater. This does not mean that they eat fish and other marine products at the exclusion of other forms of meat such as beef, pork, chicken, duck, or meat from other land animals. In fact, the Asian in general, and the Chinese in particular, are renowned for their taste for animal meat, including many exotic animals. However, the culture of raising land animal for meat, rather than for their labor (e.g., cows, buffalos, elephants, horses), is relatively more recent, and, by far, the most commonly consumed form of protein is fish. The main sources of fish and other aquatic

Table 1
Examples of Popular Freshwater and Marine Fish in Thailand and Their Fat Content

Common name	*Local name*	*Scientific name*	*Fat content, g/100 g*
Freshwater fish			
Common silver barb	Pla Ta-pien	*Puntius gonionotus*	4–8
Nile tilapia	Pla Nil	*Oreochromis niloticus*	<2
Spotted featherback	Pla Graai	*Notopterus chitala*	
Snake skin gourami	Pla Sa-lid	*Trichogaster pectoralis*	4–8
Striped catfish	Pla Sa-waai	*Pangasius sutchi*	8–9
Striped snake-head fish	Pla Chon	*Channa striatus*	8–9
Swamp eel	Pla Lai	*Fluta alba*	<2
Walking catfish	Pla Duck-oui	*Clarias macrocephalus*	14.7
Marine fish			
Black-banded trevally	Pla Samlee	*Seriolima nigrofasciata*	9.2
Black pomfret	Pla Jalamet Dum	*Parastromateus niger*	2–4
Silver pomfret	Pla Jalamet Khao	*Pampus argenteus*	4–8
Grouper	Pla Gow	*Epinephelus sexfasciatus*	<2
Giant seaperch	Pla Ga-pong Khao	*Lates calcarifer*	2–4
Malabar red snapper	Pla Ga-pong Dang	*Lutjanus malabaricus*	<2
Short-bodied mackerel	Pla Tu	*Rastrelliger brachysoma*	<2
Spanish mackerel	Pla In-see	*Scomberomorus commerson*	<2

Adapted from refs. *14–16*.

animals are the seas, paddy fields, freshwater ponds, rivers and lakes. Some countries (e.g., Thailand, Vietnam and Indonesia), with long coastlines and maritime experiences, have a relatively big fishing industry, both coastal and deep sea. Thailand, in particular, has a large seafood processing industry and is constantly in search for raw materials from the other countries in the region. More recently, as a result of the diminishing catches from the seas and other natural freshwater sources, aquaculture, either in fresh, brackish, or saltwater, has become a big industry in most SE Asian countries. Thailand, Vietnam and Indonesia, for example, have become major world producers of shrimps. In fact, raising other animals for meat and/or milk (e.g., cows, pigs, chickens, ducks, goats, buffalos), especially for export market, has also become a big business in this region, with the usual economic (i.e., over or under production, price), health (e.g., avian flu, foot and mouth disease, SARS), social (e.g., smell, noise), cultural (e.g., meat eater, vegetarian), and religious (e.g., cows in the Hindu religion and pigs in the Muslim) problems it normally entails.

Many types of fish and other seafood are consumed in many different forms in SE Asia. Table 1 below lists some of the most popular freshwater and marine fish in Thailand and their lipid content.

Aside from fresh consumption in the forms of steamed, fried, or curried dishes, fish and other seafood are traditionally processed or preserved in many other forms. These include cooking in soy sauce, comminuting (e.g., fish or shrimp balls, fish jelly products, fish fingers), curing (e.g., salted but not dried, pickled), drying (e.g., salted and dried or semi-dried), fermenting (e.g., fermented fish, fish paste, fish sauce), powdering

or flaking (e.g., plain, salted or sweet), smoking (e.g., smoked fish or oyster), others (e.g., fish or prawn crackers, barbecued fish) *(17)*. Of course, canning, freezing and surimi production are now common in modern fish industry, producing products essentially for export markets.

Freshwater fish generally contain higher levels of C_{18} polyunsaturated fatty acid (PUFA) as well as 20:5ω-3 eicosapentaenoic acid (EPA) and 22:6ω-3 docosahexaenoic acid (DHA) as compared with marine fish *(18–21)*. The fatty acid composition of freshwater fish is also high in ω-6 PUFA, especially linoleic acid and arachidonic acid. The ratio of total ω-3:ω-6 fatty acids for freshwater fish ranges from 1 to about 4, which are much lower than for marine fish.

In recent years, much research has been focused on fish oil and ω-3 fatty acids on their protective role against the development of cardiovascular diseases and rheumatoid arthritis *(22)*. Coronary heart disease (CHD) has been identified as a leading cause of death in many countries including those in SE Asia. In Thailand, the mortality rate of CHD has been increasing every year *(23)*. Therefore, greater consumption of fish, both freshwater and marine, is being encouraged. However, not all fish are equal in their lipid and ω-3 fatty acid contents. The above Table gives some indication of the variability of the lipid content among eight freshwater fish and eight marine fish.

1.4. Fruits and Vegetables

With the climate ranging from tropical to sub-tropical, high monsoon rainfall, and fertile soil, the region is teeming with all kinds of vegetation. In most SE Asian villages, one needs only to walk down to the bushes around one's house to find enough vegetable for one's meal. Most village household would plant some fruit trees in the yard, for its own consumption as well as for sale or bartering. The level of consumption of fruits and vegetables in SE Asia is, therefore, generally high. Most side dishes eaten with rice normally contain some vegetable, or even fruit. Fruits, either raw or cooked into desserts are normally eaten after the main meal, and SE Asians generally love their desserts after a meal, though the pressure of time has made this less so in big cities.

Common tropical fruits include banana, cantaloupe, dragon fruit, durian, fig, jackfruit, java apple, longan, longong, lychee, mango, mangosteen, melon, orange, rambutan, palm fruit, papaya, persimmon, pineapple, pomegranate, pomelo, sapota, soursop, star fruit, sweetsop, watermelon, some with many different cultivars. Though smallholders grow most of the fruits in SE Asia, commercial scale orchards are now quite common, particularly in Thailand. Fresh-cut fruits are usually served with meals, especially breakfast, in most hotels. Many of these fruits are now exported in fresh or processed forms around the world.

A great variety of vegetables are grown and consumed in SE Asia. These include common vegetables such as asparagus, bean, beet, broccoli, cabbage, carrot, cauliflower, celery, corn, cucumber, lettuce, mushroom, onion, pepper, potato, pumpkin, spinach, squash, tomato, and more exotic varieties such as bamboo shoot, bean sprout, bitter gourd, bok choy, Chinese celery, egg plant, kai lan, lady finger, long bean, morning glory or water convolvulus (kang kong), palm shoot, shallot, tamarind, taro root, water chestnut, water crest, and yam. Fresh or boiled vegetables are often eaten as a side dish with spicy sauces (e.g., *Nam Prik* in Thailand and *Sambal Belachan* in Brunei, Malaysia, Singapore and Indonesia).

Two common preservation methods for fruits and vegetable, (i.e., drying and pickling), are widely used in SE Asia to preserve the highly perishable produce for use during the dry season. Sun drying is the most commonly used method in rural areas. However, the product quality is rather inconsistent since it is highly dependent on the elements, which cannot be strictly controlled. Generally, sun drying does not lower the moisture content of the product below 15%, which is too high for storage stability *(24)*. Much of the nutrients, especially vitamins and some phytochemicals (e.g., β-carotene, lutein, and zeaxanthin) are destroyed during the lengthy drying process *(25)*. Without proper packaging, the product may be infested with insects and microorganisms, giving rise to further quality deterioration or potential health hazard. Modern dehydration methods, (e.g., cabinet or freeze-drying), are now available in the food industry, but the products are mainly for export markets and are generally too expensive or too alien to average SE Asian consumers who prefer fresh produce.

Fruit and vegetable pickles are produced and consumed in large quantities in SE Asia. Salt pickling is the most common method for fruits such as lime, mangoes, olive, peach, plum, and vegetables such as cabbage, Chinese Choy, cucumber, morning glory, mustard green. For some fruits (e.g., mango), sugar is added to the pickled products to make them more appetizing. Fruit pickles are normally eaten as snacks or after the main meals; or some (e.g., lime or peaches), may be used in soup. Vegetable pickles are consumed as a side dish, or with rice porridge. Pickling adds nutrients and greater storage stability to fresh fruits and vegetables by stimulating the growth of the beneficial Lactobacillus, which produce lactic and citric acids. These acids, together with salt, prevent the growth of spoilage microorganisms *(24)*. Several strains of *Lactobacillus* have been identified as probiotics, which can displace toxin-producing microorganisms in the gastrointestinal tract and produce metabolites that are beneficial to health *(26,27)*. Some of the strains can also produce antimicrobial compounds such as bacteriocins, which can prevent the growth of some pathogens *(26,28)*.

Fruits and vegetables provide a considerable portion of carbohydrates, especially sugars and fiber. They are also excellent sources of some vitamins, minerals and, importantly, phytochemicals with biological activity that have been shown capable of prevention of cancer, heart disease and many other diseases *(29)*. Not all fruits and vegetables are equal nutritionally or medicinally, and these values are also dependent on how they are processed, stored and prepared for consumption. Table 2 gives some examples of fruits and vegetables common to SE Asia, their bioactives, and diseases or health conditions they have been found to be effective against.

1.5. Spices

It may not be an over-exaggeration to suggest that the discovery of the New World resulted partly from the European's appetite for spices. The colonization of many Asian countries was also to secure the rich supply of spices and other natural resources in these countries. The Dutch colonization of Indonesia is a good case in point. Many sought-after spices originated or are grown extensively in SE Asian countries like Indonesia, Malaysia, Myanmar, the Philippines, Thailand, and Vietnam. Among these, are basil, chili, cinnamon, clove, galangal, garlic, ginger, kafir leaf, lime grass, marjoram, mint, nutmeg, oregano, pepper, rosemary, sage, sweet basil, thyme, turmeric, to name

Table 2
Examples of Southeast Asian Fruits and Vegetables, Their Bioactive Components, and Their Uses or Diseases and Health Conditions They Have Been found Effective Against

Type of fruit and vegetable	*Bioactive/nutrient*	*Disease/health condition/use*
Fruit		
Banana (Thai: *Kluay Nam Wa*)	β-sitosterol, campesterol, stigmasterol, fiber, minerals (Ca, Fe, K), vitamins	Diarrhea
Grape, red	Resveratrol and many other antioxidants	Lower amyloid-β peptides that cause the plaques in the brain leading to Alzheimer's, Huntington's, Parkinson's and prion diseases
Guava	Lycopene, vit. A, Fe, Ca	Antitumor, anti-inflammatory, oral health, cold and flu
Mango	β-carotene, β-cryptoxanthin	Chemopreventive, anti-tumor
Mangosteen	Xanthones (α-mangostinin and polyphenol, standardized to 3500 μmole TE/g ORAC)	Arthritis, asthma, eczema, fatigue, gastritis, irritable bowel, muscle or joint pain, urinary tract infection
Papaya	β-carotene, vit. B_1, B_2, C, Ca, Fe, P	Laxative, digestive aid
Pineapple	α-tocopherol, retinal	Diuretic, kidney stone, anti-inflammatory, ulcer
Pomegranate	Polyphenols, anthocyanin	Osteoarthritis, heart disease, hypoxia ischemia-related brain injury in baby
Vegetable		
Carrots	Falcarinol	Reduce cancer in rats by 1/3
Cruciferous (broccoli, cabbage, cauliflower, mustard)	Allyl-isothiocyanates, isothiocyanates	Bladder, breast, lung, prostate, stomach cancers
Chili pepper	Capsaicin, citric acid	Chemopreventive
Eggplant	β-sitosterol, stigmasterol, vit. A, B, and C, Ca, Fe, K, P	Reduce cholesterol and blood pressure, Alzheimer's, anti-inflammatory, urinary complaints
Morning glory (Convolvulus)	β-carotene, gibberellin, quercetin, α-tocopherol, tyramine	Eye health, diabetes, anti-inflammatory, skin disease
Pumpkin	β-carotene, β-cryptoxanthin, β-sitosterol, vit. C, phosphorous	Skin tumor, urinary complaints, kidney stones, antirheumatic, ulcer
Tomatoes	Chlorogenic acid, lycopene, polyphenols	Breast, pancreatic and prostate cancer, age-related diseases

Adapted from refs. *30–35*.

just a few. These spices have not only made plain food more interesting and enjoyable, but have quite often imparted their erstwhile-unexpected nutritional and medicinal goodness to the consumers as well.

There has been a worldwide renaissance of interest in herbs and spices in recent years for their nutritional and medicinal properties. Consumers are becoming increasingly aware of the widespread use of chemicals and synthetic products in their food and medicine, at times with disastrous consequences. Thus, the "back to nature," the "organic," or the "alternative" lifestyle has found an expanding sphere of followers during the past few decades. Indeed, research in various countries have shown that natural compounds in these herbs and spices do have important nutrients and/or healing properties that are of distinct benefit to the consumer. Of course, more research is needed to fully explore the potential benefit these materials have to offer. Therefore, a review of the SE Asian diets cannot be complete without understanding the influence of the spices the use of which is so pervasive in their culinary practices.

The use of spices in SE Asian cooking varies considerably among the countries and ethnic groups, depending on whether they are influenced by the Malaysian, Indian, or Chinese cuisine, and also on their socioeconomic background. Malaysian and, especially, Indian curries incorporate a lot of dry spices, pieces or powder, into the ingredients. Coconut milk is also used to thicken and enrich the curries, which may be fried or slow cooked to a thick paste. Those with the Chinese and other ethnic influences use less dry spices but more of fresh herbs in their dishes. Little coconut is used to enrich their food, and most dishes are either stir-fried or cooked in lighter broth or soup. Those in a higher socioeconomic scale tend to enjoy more elaborate meals, using better and greater variety of ingredients than their poor counterparts who have to make the best from whatever they can afford. Quite often, however, a simple and inexpensive dish prepared by a talented village cook, with the help of delicately balanced herbs and spices added to the basic ingredients, can be very delicious.

Not uncommonly, the use of herbs and spices can make or break a meal. Too much hot chili or too strong a flavor of turmeric or basil, for example, may turn a more sensitive palate off the dish. It should be noted that the use of herbs and spices over the centuries has been primarily to enhance the taste and flavor of the food. However, the unexpected added nutritional or medicinal value of these plant materials have certainly raised the profile of these ingredients to a more prominent level where they now capture the attention of a wider culinary audience. The value of herbs and spices is in their volatiles and soluble bioactive components, therefore, the method of food preparation is extremely important in determining how best to get the most value out of them. Obviously, the amount of the bioactive compounds from herbs or spices leached out into the food during cooking is very small, and the types of compounds extracted out depend also on whether it is cooked in water or fried in oil and on the cooking temperature. Therefore, the quantity of the bioactives ingested from each meal may be just a fraction of their effective minimum dosages. Nevertheless, when a variety of herbs and spices are consumed at almost every meal the cumulative quantity may be sufficient to offer long-term benefits to the consumer. The following (Table 3) are some examples of common herbs and spices used in SE Asian cooking, their main bioactive components, and some of their uses, or diseases and health conditions they have been found to be effective against.

Table 3
Examples of Herbs and Spices Used in Southeast Asian Cooking, Their Main Bioactive Components, Their Uses or Diseases, and Health Conditions They Have Been Found to be Effective Against

Herb and spice	*Bioactive*	*Disease/health condition/use*
Chili	Capsaisin	Respiratory system, blood pressure and heart disease, digestive system (used as stomachic, carminative and antiflatulence agent)
Cumin	Apigenin glucoside, ascorbic acid, cuminaldehyde, limonene, linalool, thymol	Heart disease, gastrointestinal disease, immune system, kidney stone, cancer, antimicrobial
Galangal	Cineole, α-pinene, bornyl acetate, geranyl acetate, β-farnesene, β-bisabolene	Antifungal, antimicrobial, antitumor, antiulcer, antimutagenic
Garcinia cambogia (Som Khaek)	Hydroxycitric acid (HCA)	Reduce glucose absorption, diabetes, aid weight loss
Garlic	Allicin, alliin, diallyl sulfide, thiosulfinates, saponin, caffeic acid	Antimicrobial, diaphoretic, diuretic, expectorant, antiflatulence, cholesterol lowering agent
Ginger	Gingerol, alanine, ascorbic acid, β-carotene, β-sitosterol, caffeic acid, chlorogenic acid, curcumin, ferulic acid, kaempferol, quercetin, selenium, shikimic acid, terpinene	Carminative, antinauseant, antiflatulence
Kaffir lime	Aviprin, borneol, δ-cadinene, camphor, citronellal, α-eudesmol, geraniol, limonene, linalool, menthone, α-pinene, rutin, β-sitosterol, α-terpinene	Appetizer, cold, fever, gastrointestinal conditions
Lemongrass	Saponin, β-sitosterol, hexacosanol, triacontanol, citral, myrcene, linalool, α-terpineol, geraniol, citronellol	Antimicrobial, diuretic, emmanagogue, antiflatulence, antiflu
Pepper	Piperine, piperidine, piperettine, piperolein, piperanine, monoterpenes, sesquiterpenes	Carminative, antipyretic, diaphoretic, diuretic, antiepileptic
Sweet basil	Methyl chavicol, linalool, camphor, eugenol	Carminative, diaphoretic, expectorant, digestant, stomachic, mouthwash
Turmeric	Curcumin, turmerone, zingiberene	Inhibit melanoma cell growth and kill tumor cells, Alzhimer's disease, carminative, antiflatulence, stomachic

Adapted from refs. *36–43*.

Table 4
Estimated Population, Life Expectancy, and Fertility Rate Among Southeast Asian Countries

SE Asian country	*POP (×1000) 2000 estimate*	*Life expectancy*		*Fertility rate*	
		1965–1970	*1995–2000*	*1965–1970*	*1995–2000*
Brunei Darussalam	328	64.9	75.5	5.9	2.8
Cambodia	11,168	45.4	53.4	6.2	4.6
East Timor	885	37.5	47.5	6.2	4.4
Indonesia	212,107	46.0	65.1	5.6	2.6
Laos	5,433	40.4	53.2	6.2	5.8
Malaysia	22,244	59.4	72.0	5.9	3.2
Myanmar	45,611	47.4	60.1	6.0	2.4
Philippines	75,967	56.2	68.3	6.0	3.6
Singapore	3,567	67.9	77.1	3.5	1.7
Thailand	61,399	56.7	68.8	6.1	1.7
Vietnam	79,832	47.9	67.4	6.0	2.6
Total	518,540	47.8	65.7	5.8	2.7

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2. THE FOOD OF SOUTHEAST ASIA

It has been said that it is impossible to starve in SE Asia, unless one intends to do so. This is because the region is so blessed with plentitude of materials in its natural habitats that one can simply go out and harvest enough food ingredients for the preparation of any of his/her meal. King Ramkamhaeng the Great of the first Siamese Kingdom around the 13th Century recorded on a stone tablet, with the first Thai alphabets which he invented, that: "There are fish in the waters and rice in the fields, and whomsoever wish to pursue trades of any kind in this Kingdom may do so." This indicated the abundance of the land that could support anyone willing enough to put even the minimum of effort to help oneself. Therefore, food is not a problem in SE Asia; however, how well one is fed and with what quality of food depends also on other factors that affect one's quality of life.

2.1. Population, Life Expectancy, and Fertility Rate

One common yardstick to measure how well people in any country live with respect to diet, healthcare, environment, and general quality of life is their life expectancy. Table 4 compares life expectancy and fertility rate among the SE Asian countries as reported by World Health Organization (WHO) *(44)*. The Table shows the general improvement in both categories in all countries between the periods of 1965 and 1970 and 1995 and 2000, though at different rates depending on their level of development. Please note that East Timor was a part of Indonesia until it gained independence in August, 1999.

It is noteworthy that the two smallest countries, Brunei and Singapore, with the highest standard of living, have the longest life expectancy of 75.5 and 77.1 yr, respectively. Singapore also has the lowest fertility rate of 1.7 children/100 persons. Malaysia, with its rapid rate of development, to be a fully developed nation by the year 2020, has the 3rd longest life expectancy of 72.0, but its fertility rate is still relatively high at 3.2. Thailand comes a distant 4th after Malaysia with 68.8 yr of life expectancy, but with the fertility

rate equal that of Singapore. As to be expected, East Timor, Laos and Cambodia, the three least developed SE Asian nations, fare the poorest in both respects, with life expectancy of 47.5, 53.2 and 53.4 yr and fertility rate of 4.4, 5.8 and 4.6%, respectively.

Obviously, diet is not the only reason for the length of life expectancy. General quality of life, which is closely related to income, healthcare standard, and living environment, also plays very important roles in determining how well and how long people live. Even within one country there is a wide discrepancy in life expectancy among the citizens, dependent on their individual standard of living. Therefore, in reviewing the types of food and dietary habits of each SE Asian country, it will be a mistake to try to relate them directly to the general state of health and well-being of all its citizens. In this Chapter, the review will focus on the major types of food, or dishes, enjoyed by the people of different countries or racial groups, their ingredients, methods of preparation, and the times or occasions they are consumed. Where possible or appropriate, the dietary value and possible effect on health of the dishes or their ingredients will be evaluated. The types of food will be loosely grouped under country or group of countries or ethnic groups of origin. As stated earlier, SE Asia is a melting pot of races, therefore, there is bound to be overlaps, or similarities among their dishes.

2.2. Type of Food Outlet

It should be noted that, apart from home cooking, one could obtain food in Southeast Asia through several types of outlets. The type of outlet one chooses usually depends on one's socioeconomic status, where one lives, one's favorite cooks or dishes, time constraint, convenience, and the occasion for the meal.

2.2.1. Hawker Food

For those on low budget, hawker-style delivery can be found almost anywhere in cities or villages. The hawkers' carts are, in fact, mobile kitchens, which deliver the hawkers' specialty foods such as noodles (e.g., fried or soup), rice and food (e.g., chicken rice, curry on rice, fried rice), satay meats with sauce, soymilk or soybean curd products, fried bananas or other fruits, various kinds of local desserts. The carts may be pushed from place to place along their regular routes, or remain on the same favorite spots. The food is usually prepared fresh on the spot, or pre-prepared and kept in closed containers till served. They are, perhaps, the quickest and cheapest way to get a meal, either for breakfast, lunch, or dinner. The main drawback for this type of food outlet is that the standard of hygiene is not always the best or most reliable. This results from the fact that the operators may be good cooks (i.e., they have to be or they will not last long in the business.), but they are certainly not professionally trained chefs who understand the basic principles of sanitation and causes and prevention of food poisoning. Also, because of the nature of the hawker business where carts are normally on the streets, away from convenient supplies of washing or drinking water, and the carts are constantly exposed to the elements, one has to be very careful in choosing a hawker whose standard of quality and safety one can trust. A lot of food poisoning incidences in these countries may be traced back to hawker foods. Fortunately, the Ministry of Public Health in some countries has begun to appreciate the extent of public health hazard caused by this type of food outlets and has instituted training and inspection programs to alleviate or minimize the problems.

2.2.2. Roadside Restaurant

Roadside restaurants are another type of food outlet commonly found in Southeast Asia. This type of outlet usually means small shops in the village, town, city, or along the highway, which can be seen and are accessible from the road. They serve relatively simple food such as noodles or curries with rice, or other dishes ordered from a limited menu. They may also serve various kinds of beverages or snacks. Customers are workers, tourists, or students who do not want to spend too much money or time on their meals. They are more organized than the hawkers, with a small kitchen, a cook and a helper or two to help prepare and serve the food. Sanitary standards are definitely better than the hawker-style operations, often with some basic washroom facilities. Food quality varies from barely tolerable to highly delicious, in which cases customers will travel from far and wide to taste their fares. Many large, more expensive restaurants have such a modest beginning.

2.2.3. Up-Scale Restaurants and Fast Food Outlets

Then, of course, there are up-scale restaurants and fast-food outlets in the cities or outskirt of the cities of Southeast Asia, just like anywhere else in the world. They specialize in major ethnic food such as Chinese, Thai, Malaysian, Indian, Vietnamese, or even serve Western-style menus. Seafood restaurants serving a huge variety of dishes prepared from fresh seafood have become very popular in recent years. Most of these restaurants have international standards of quality and safety and, therefore, are more expensive than the previous two types of outlets. Their customers are business people, office workers, tourists, and more well to do families. It is often said that eating is a big business in Southeast Asia, and this is certainly borne out by the number of customers frequenting some of these popular restaurants on a daily basis. Countries like Thailand have taken advantage of the increasing popularity of their ethnic foods by facilitating the expansion of their ethnic restaurants in foreign countries. The Thai government, for example, has been promoting Thailand as a kitchen of the world and supporting training programs for Thai chefs to be sent to Thai restaurants overseas.

2.3. Food of Brunei, East Timor, Indonesia, Malaysia, and Singapore

With the exception of East Timor and Singapore, the majority of people in these countries are Muslim. East Timorese are mainly Christian, with Muslim as the second largest group. The biggest ethnic group in Singapore is Chinese, followed by Malays and Indians, but with a strong influence from Western culture. Therefore, the main diets in these countries consist of spicy Malaysian, Indonesian, and Indian dishes and a milder Chinese cuisine, depending on the racial composition of the communities. Of course, one can find McDonald's, KFC, or any major franchised American restaurants in most big cities these days, but native dishes are still the most common fare among the locals.

People in these countries traditionally divide food into four types (i.e., heating, cooling, neutral, and clarifying) *(46)*. A natural balance between these categories of food is very important for one's good health and well-being. Heating foods are those needed for the growth and maintenance of the body. They include meats such as beef, mutton and goat, fried foods like fried banana or plantain, curry puffs, curries, and stews. They tend to raise body temperature, and over-consumption may make one feels uncomfortable.

Cooling foods, by contrast, include most fruits and leafy vegetables (e.g., mangosteen, Chinese pears, okra [lady's fingers], lettuce and cucumber). They also include tea, barley water, and sago. Their consumption tends to lower body temperature, settle the stomach, and make one feel more comfortable. Neutral foods include chicken and most fish. Their consumption nourishes the body without unduly raising or lowering its temperature. The clarifying foods, such as herbal tea or soup, are those designed to remove toxins from the body and keep it functioning normally. They should be eaten twice monthly to purify the blood.

Listed in Table 5 below are some of the most commonly known and consumed dishes in these countries, their ingredients and brief cooking methods.

Note that Southeast Asian dishes, indeed most other Asian dishes, are prepared primarily with their sensory rather than their nutritional quality in mind. In addition to salt, sugar and sauce, herbs and spices are added for their ability to enhance the flavor and the taste of the food. Not until recently have studies revealed the extraordinary nutritional and/or medicinal properties most of these herbs and spices possess. Therefore, many Asian dishes have become internationally popular not only for their excellent taste and spiciness but also for their "healthy" image. Indeed, this image appears to have been based on a considerable amount of proof, from the rarity in obesity and generally low incidences of major diseases such as cardiovascular disease and cancer among the Asian populations, as compared with those in Europe and America. Not until the introduction of Western-style food, especially fast food, into these countries in the past 30 yr that the incidences of these diseases and obesity have begun to rise. In Thailand, for example, the prevalence of overweight and obesity among children and adolescents has increased dramatically during the past 20 yr and is more pronounced in children from private schools and urban communities than in those from public schools or rural areas. Results from two national surveys in 1991 and 1996 indicated that the problem of overweight and other risk factors for cardiovascular disease among adults have increased significantly. The diet-related chronic degenerative diseases of the circulatory system have become the number one cause of death in Thailand whereas cancer has ranked number three since the late 1980s *(47)*.

Most of the dishes listed in the following Table are "heating" foods because they contain some kind of meat. *Char Kuay Teo*, *Laksa Penang*, and *Malu Abulthiyal* may be classified as "neutral" food. A cursory look at their lists of ingredients, many of which include lard and coconut milk, one may be inclined to consider them unhealthy. However, essentially all dishes contain a number of herbs and spices, and some may have added vegetables. These ingredients may, to varying degrees, neutralize or reduce the nutritionally negative properties of meat, lard or coconut. Furthermore, most of these foods are accompanying or side dishes to be consumed with the main staple (i.e., rice). Therefore, they are not normally consumed in great quantity at any meal. For the main meals (i.e., lunch and dinner, especially the latter), there will usually be more than one accompanying dish, some of which consist principally of vegetables or some fruits. Some pickled vegetables and/or fruits, such as Malaysian *Achar* (spicy pickled mixed vegetables) or *Ard Jard* (sweet and sour cucumber salad usually eaten with *Satay*), may also be consumed during these meals. In addition, cooling or clarifying drinks such as barley water, pennyworth juice, chrysanthemum or other herbal tea may also be enjoyed to round off the meals. On the whole, therefore, most SE Asians try,

Table 5
Some Commonly Known and Consumed Dishes in Brunei, East Timor, Indonesia, Malaysia, and Singapore—Ingredients Used and Brief Cooking Methods

Name of dish	*Ethnic origin (Time consumed)*	*Ingredient*	*Method of cooking*
1. *Buk Kut Teh* (Spicy sparerib consommé)	Malaysian Chinese (Breakfast or lunch)	Beef, mutton or pork sparerib, sugar, lard, salt, pepper, clove garlic, preserved brown and black soybean, cinnamon bark	Cook in soup
2. *Beriani*	Indian (Anytime)	Chicken, beef or mutton, clove garlic, sliced shallots, curry powder, salt, chili, grated coconut, ginger, ghee, cloves, cinnamon stick, cashew nuts, almonds, long-grain rice	Ingredients fried in oil then mixed with cooked rice
3. *Char Kuay Teow* (Fried flat rice noodles)	Chinese (Anytime)	Lard, eggs, garlic, flat rice noodles, bean sprouts, dark soy sauce, chili sauce, Chinese sausages, chives, cockles	Fry in oil
4. *Laksa Penang*	Malaysian Chinese (Anytime)	Garlic, lemongrass, turmeric, dried chili, chili paste, shrimp paste, sugar, phaeomaria (*Bunga Kantan*), polygonum (*Daun Kesom*), tamarind, rice vermicelli, wolf herring (*Ikan Parang*)	Cook in soup
5. *Malu Abulthiyal*	Indian (Lunch or dinner)	Halibut, salmon or tuna, goraka, salt, black pepper, turmeric, spring curry leaves, rampe, garlic, roasted curry powder	Fry in oil
6. *Murtabak* (Meat crepes)	Indian (Anytime)	Egg, wheat flour, salt, pepper, mutton, turmeric powder, diced onion, cardamom seeds, roasted coriander seeds, aniseeds	As for crepes
7. *Rendang* (Curried meat)	Malaysian/Indonesian (Eaten with rice for lunch or dinner)	Shrimp paste, rump steak (beef, chicken or mutton), lemongrass, salt, sugar, soy sauce, grated coconut, galangal, candlenuts, curry powder, chili, shallots, garlic, thick coconut milk	Cook meat in the sauce of all other ingredients
8. *Satay* (Barbecued meat with peanut sauce)	Malaysian/Indonesian (Snack or full meal for lunch or dinner)	Meat (beef, chicken, mutton, goat); Marinade for meat: shallots, garlic, turmeric, lemongrass, galangal, coriander seeds, dark soy sauce, salt, sugar, oil; Peanut sauce: shallots, garlic, lemongrass, dried chili, chili paste, galangal, salt, sugar, lime juice, tamarind paste, fresh roasted ground peanuts, oil	Marinate meat and barbecue; peanunt sauce ingredients are cooked together

Reprinted with permission from ref. *46*.

either subconsciously or through traditional eating habit, to balance their diet with different categories of food.

2.4. Food of Cambodia, Laos, Myanmar, the Philippines, Thailand, and Vietnam

The grouping of the foods in these six Southeast Asian countries is based principally on the fact that their diets have a stronger Chinese influence rather than the Malaysian, Indonesian or Indian dominance as in the previous five countries. The spices used are generally milder and less coconut milk is used than in Malaysian or Indian cooking. Nevertheless, there is a considerable difference among the six styles of cuisine, due mainly to the variation in the cultural and economic structure of these countries.

2.4.1. Cambodia

Cambodian cuisine is said to have both Chinese and Thai influences. Their food is more akin to Thai food but without the spiciness *(48)*. The main national staple is rice, but French colonial influence has brought the appetite for bread, especially French-style baguettes, to Cambodia than any other Southeast Asian country. Freshwater fish and prawns and seafood from the Gulf of Thailand are very popular. Other forms of meat are also available but generally more expensive than fish. Soup, such as sour fish soup or sour and spicy prawn soup (*Somlar Machou Banle* or *Bangkang*, the latter similar to Thai *Tom Yam Gung*), is very popular and served at almost every meal. Other common Cambodian dishes include *Khao Poun* (rice noodles in a coconut-based sauce), *Hamok* (fish with coconut milk steamed in a banana leaf), *Sach Mon Chha Khnhei* (stir-fried chicken with ginger), *Somlar Machou Sachko* (sour beef stew), and *Choeeng Chomni Chrouc Chean* (fried pork spareribs).

2.4.2. Laos

Lao cuisine is distinctive and is quite similar to that of the ethnic northeastern Thai. About 90% of the Laotians eat glutinous or sticky rice, not the common long-grain variety *(49)*. Glutinous rice is generally served with a selection of dips, parboiled vegetables, vegetable or meat salad, soup and various curried meat or fish dishes. Laotian dishes are generally cooked with fresh ingredients that include vegetables, chicken, duck, pork, beef, and water buffalo. Fish and prawns are readily available but are nearly always of freshwater varieties, because Laos is a landlocked country *(50)*. Popular Laotian dishes include *Tam Som* (grated green papaya salad, like Thai *Som Tam*), *Laap* (spicy minced meat same as Thai *Larb*) and *Tom Khaa Kai* (chicken soup with galangal and coconut milk, similar to a Thai dish of the same name).

2.4.3. Myanmar

Myanmar food may also be ranked between Chinese and Thai food, somewhat richer than the former, but less spicy than the latter *(51)*. The basic Burmese food is mainly rice and curry. Some Burmese curries are spicy and there is always fish paste in many forms with salad. Soup, mostly made of vegetables, is taken together with rice and other dishes. *Mohinga* (rice noodles with fish gravy) is a favorite for breakfast. *Ohn-No-Khaukswe* (noodles with coconut chicken gravy) is the most popular among Burmese foods. Favorite desserts are *Sanwin-ma-kin* (sweet cakes), Myanmar-style banana cakes, *Kyaukchaw* (seaweed jelly) and gagger.

2.4.4. The Philippines

The Philippine culture is strongly influenced by both the East (e.g., Chinese, Malay) and the West (e.g., Spanish, American). With 92% of the population being Christian (83% Roman Catholic), 5% Muslim, and 3% Buddhist, their culinary style may be viewed as a unique fusion of a multicultural hotpot. Filipino food is a combination of sweet, sour, and spicy tastes. Spices are commonly used in their cooking, but while other Asians prefer subtlety and balance in their use of spices the Filipinos prefer to savor their flavors at once *(52)*. Dishes range from a simple meal of fish and rice to rich *Paellas* and *Cocidos*. Popular dishes include *Lechon* (whole roasted pig and calf), *Longanisa* (sausages), *Tortas* (egg wraps), *Pan de sal* (bread rolls), *Adobo* (chicken and/or pork marinated in garlic, soy sauce, and vinegar), *Kalderetang Kambing* (rich goat stew), *Kare-kare* (ox tail cooked in peanut sauce), *Sinigang* (sour soup), *Pancit* (stir-fried noodles), *Lumpia* (fresh or fried spring rolls) and *Halo-halo* (a cold exotic fruit mix dessert).

2.4.5. Thailand

Thai cuisine has become quite popular in the past 20 yr, with Thai restaurants sprouting up all over the world. Thai food is known for its healthy, low cholesterol, and low fat content as for its spicy taste and elegant presentation. The Thai uses less spices and more fresh herbs than the Malay or Indian. Thai cuisine emphasizes a subtle and harmonious blend of spicy, sweet and sour tastes to equally satisfy the nose, eye, and palate. With the increasing popularity of Thai delicacies, the formerly little known herbs and spices like *Kha* (galangal), *Ka Min* (turmeric), *Makrut* (kaffir lime) and *Takrai* (lemongrass) have appeared on supermarket shelves across Europe and America *(53)*.

Thailand is divided into four major regions (i.e., North, Northeast, Center, and South), each with its own distinctive culinary style. Central cuisine is, perhaps, the most well known to the outside world, a fusion of the local, Chinese and Western styles of cooking to become uniquely Thai. Central dishes are milder as compared to those from the Northeast or the South, the popular ones being *Tom Yam* (sour and spicy soup with shrimp or fish), *Kaeng Kheaw Waan* (green curry with chicken or beef), and *Tom Khaa Kai* (chicken in galangal and coconut soup). Northern cuisine has strong Chinese and Burmese influences with a widespread use of tomatoes as a common ingredient. Popular Northern dishes are *Khao Soy* (spicy noodles in chicken or beef broth), and *Kaeng Hang Lay* (curried pork with ginger and peanuts). Northeastern dishes are quite similar to those of Laos, consisting of spicy *Larb* (minced pork or beef or fish with chili, roasted rice, fish sauce and fresh mint) and *Som Tam* (sweet and spicy papaya salad), served with glutinous rice. Southern cooking uses more coconut milk than the other regions, influenced by the Malaysian taste. Thus, their curries are richer and creamier (e.g., *Kaeng Matsaman* [beef curry in a thick coconut cream sauce with peanuts, potatoes and chopped red onions], *Kaeng Panaeng Kai* [savory chicken and coconut curry], and *Kaeng Som* [hot and sour fish ragout with vegetable]). *Naam Prik* (salty shrimp paste pound with garlic, sugar, chili and lime juice) is almost always served with the main meals as a dip for fresh or cooked vegetables. Desserts or fresh fruits usually round off the Thai meals to tone down the spiciness of the main dishes.

2.4.6. VIETNAM

Vietnamese cuisine reflects the influences from China, Cambodia and France. Like other SE Asian countries, the main staple is rice, but bread, especially baguettes, made popular by the French, can be found anywhere *(54)*. The Vietnamese use a lot of fresh herbs such as sweet basil, coriander and mint, and vegetables such as bean sprouts, lettuce, and cucumber, in their cooking. Popular dishes include *Cha Gio* (spring rolls of minced pork, prawn, crabmeat, fragrant mushrooms and vegetables wrapped in thin rice paper and deep fried, eaten with lettuce leaf, fresh mint and other herbs, and a sweet sauce), *Cuon Diep* (shrimp, noodles, mint, coriander and pork wrapped in lettuce leaves), *Banh Khoai*, or "Hue pancake" (batter of rice flour and corn fried with egg to make a pancake, then wrapped around pork or shrimp, onion, bean sprouts and mushrooms), and *Bun Bo* (fried beef and noodles served with coriander, onion, garlic, cucumber, chili peppers and tomato paste).

3. CONCLUSIONS

In general, the foods of Cambodia, Laos, Myanmar, the Philippines, Thailand, and Vietnam appear to be more "healthy" than those of Brunei, East Timor, Indonesia, Malaysia, and Singapore due to the more liberal use of fresh vegetables, herbs and spices. As a whole, however, SE Asian diets may be considered one of the healthiest, most delicious and balanced because of the abundant use of seafood, especially fish, as the main source of protein, and the incorporation of vegetables, fruits, herbs, and spices into every meal. In addition, eating rice or noodles as the main staple, complemented by other side dishes to make the meals more interesting and delicious, results in lighter food being consumed and easier to digest as compared to the heavier Western-style food rich in animal protein and fat and low in vegetables and fruits. Unfortunately, as these countries are becoming more developed and industrialized, the lure of the Western-style fast food and rich diets, especially among the more affluent in big cities, has, to an alarming degree, taken over the healthier eating habit and diets to the detriment of the general well-being of their citizens. It is to be hoped that with the increasing awareness of the relationship between diet and health and the push for a healthier lifestyle in the West, the deteriorating food trend in SE Asia can also be reversed before permanent damage is done.

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V The Columbus Concept: The Market Approach

37 Columbus Feed Around the World

Selection of Ingredients and Potential Influence on Future International Strategy for Crop Selection

Luc Coucke

Abstract

In the late 1980s, polyunsaturated fatty acids (PUFAs) of the ω-6 and ω-3 families were recognized as essential nutrients to human and animal health. The Columbus concept—a clearly defined proposal towards a return to the "wild-type" ω-6:ω-3 fatty acid ratio in the fat depots of modern livestock—had led to the development of the Columbus egg. This egg serves as prototype for the design of other "wild-type" animal-derived products (dairy and meat products) as well as of functional ω3 long-chain (LC)-PUFAs enriched Columbus eggs.

This chapter presents and discusses the range of raw materials that are commercially available for layer feed used in the production of the original "wild-type" Columbus eggs and the feed supplements that can be used to design a second generation of ω-3 LC-PUFAs enriched Columbus eggs.

Key Words: Feed ingredients; n-3 PUFAs; egg yolk.

1. INTRODUCTION

In the 1960s and 1970s, the correlation between blood cholesterol—considered at that time as a biochemical marker for cardiovascular diseases—and the contribution of dietary cholesterol, saturated and unsaturated fatty acids in the daily food supply was demonstrated *(1)*. A decade later, the importance of the contribution of the two families of ω-6 and ω-3 polyunsaturated fatty acids (PUFAs) to the human health in the long run started to attract scientific interest *(2)*. Today, the ratio of ω-6:ω-3 fatty acids in the diet seems to be one of the most important parameter to follow-up.

Mammals, including humans, are unable to synthesize linoleic acid (C18:2ω6 or LA) and α-linolenic acid (C18:3ω3 or ALA). Hence both fatty acids are said essential and must be present in the diet. Long-chain PUFAs (LC-PUFA) can be derived from LA and ALA, however the conversion is limited with yields typically ranging from 10 to 30% depending upon their respective concentration in the diet and other genetic, environmental, and physiological factors. The metabolic pathway is common to both families of essential fatty acids and involves a series of desaturases and elongases (*see* Table 1). The most important LC-PUFAs generated from these pathways are arachidonic acid (C20:4ω6 or ARA) of the n-6 series, eicosapentaenoic acid (C20:5ω3 or EPA) and docosahexenoic acid (C22:6ω3 or DHA) of the n-3 series *(3)*.

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Table 1
Essential Fatty Acid Metabolic Pathway

(ALA)	C18:3		C18:2	(LA)
		Δ6-Desaturase		
	C:18:4		C18:3	
		Elongase		
	C20:4		C20:3	
		Δ5-Desaturase		
(EPA)	C20:5		C20:4	(ARA)
		Elongase		
	C22:5		C22:4	
		Δ4-Desaturase		
(DHA)	C22:6		C22:5	

An alternative way to fish to providing consumers sufficient amount of ω-3 LC-PUFAs in the diet is to incorporate ALA in the feed components of modern livestock. Most domesticated animals do at least partially process ALA into ω-3 LC-PUFAs. Whereas the former is preferentially deposit into intramuscular fats and fat depots of the animals, the latter are mostly found in their tissue phospholipids. Therefore, a number of limitations applied to the design of "wild-type" animal-derived consumer products:

- In pork, genetic selection has allowed to design extremely lean meats (less than 2% intramuscular fat) which catch consumer preference. High quantities of ALA in the feed render the bacon of the loin softer, thus creating difficulties during meat-processing; yellowing of the fat is another risk.
- In dairy products, hydrogenation in the rumen inhibits the transfer of ω-3 fatty acids from the feed into milk. Some alternatives have been developed to render ω-3 sources resistant to the rumen. When the milk fat is higher in PUFAs, then the butter produced from it has a higher melting point.
- In poultry meat, the amount of PUFAs in the fat of the chicken carcass can be readily influenced by nutritional means. Therefore, broiler is a good potential source of ω-3 LC-PUFAs. Sufficient attention must be drawn to the stabilization of such meat in order to avoid early development of rancid or fishy taste and smell.
- In eggs, many studies have examined the possibility of transferring ω-3 fatty acids from layer feed to egg triglycerides and phospholipids, and the results are most promising.

2. INGREDIENTS FOR COLUMBUS FEED

The total lipid fraction of the Columbus egg is characterized by P:S and ω–6:ω-3 ratio's close to 1:1. For the sake of comparison, the lipid fraction of ordinary eggs exhibits a P:S ratio of ±1:2 and a ω-6:ω-3 ratio of ±9:1. An increase in the fat content of the layer feed results in the inhibition of the novo-synthesis of yolk fat, a natural process by which yolk fat can easily be manipulated. In designing the Columbus feed, it is critical that all raw materials of the ration be considered for their eventual influence on the P:S and ω-6:ω-3 ratio's. To that end, the most important raw materials commercially available for layer feed will be discussed. In general terms, the raw materials used for the Columbus feed greatly differ from those of a classic layer feed. A survey of the raw materials available in different parts of the world (e.g., cereals, leguminosae,

Table 2
Cereals

Wheat	Low fat content. Negative effect of NSPs neutralized by xylanases.	Suitable
Rice, course	Low fat and linoleic acid contents.	Suitable
Rice, polished	Low fat and linoleic acid contents.	Suitable
Barley	Negative effect of NSPs neutralized by β-glucanases.	Suitable
Rye	High amount of NSPs neutralized by xylanases.	Suitable
Triticale	Crossing between wheat and rye. Use of xylanases highly recommended. Low fat content *(4)*.	Suitable
Oats	High fat and linoleic acid contents.	Not suitable
Maize	High fat and linoleic acid contents.	Not suitable
Sorghum	Comparable to maize	Not suitable

The negative effect of cereal NSPs is related to their ability to bind water and to increase intestinal viscosity, thereby reducing access of nutrients to digestive enzymes.

Table 3
Leguminosae

Peas	Varieties with low tannin content. Winter varieties are less digestible than spring varieties.	Suitable Max 20%
Beans, heat treated (*Phaseolus vulgaris*)	Must be correctly heat treated.	Suitable Max 20%
White horsebeans (*Vicia faba*)	Too high level may cause drop in egg weight, resulting from ANFs.	Suitable Max 20%
Sweet Lupins	Rather high fat and linoleic acid contents.	Not Suitable

Table 4
By-Products From Mills and Peeling Mills

By-products from wheat	Low fat- and linoleic acid contents: wheat bran @ 3.5% crude fat, wheat middlings @ 3.4% crude fat, and wheat germ feed @ 4.6% crude fat. Preferably along with xylanases on account of their high content in NSPs. These raw materials also lack the adequate mesh structure for layer feed *(5)*.	Suitable Low level
By-products from maize	High fat- and linoleic acid contents: maizefeed meal @ 7,3% crude fat, maize germs @ 23,5% crude fat, and maize germmeal feed expeller @ 5,3% crude fat.	Not suitable
By-products from rice	Rice bran, regardless its levels of husks and raw ash, is rich in linoleic acid *(6)*.	Not suitable

by-products from mills and peeling mills, by-products from starch plants, by-products from sugar plants, by-products from distilleries and breweries, dried bulbs[tubers] roots, oleaginous seeds, by-products from the vegetable oil industry, by-products of animal origins, oils and fats, and dehydrated grass meal) will illustrate this (all fatty acid analyses of raw materials discussed in this article are shown in Table 2 *[20]*).

Table 5
By-Products From Starch Plants

By-products from wheat	Wheatgluten feed along with xylanases. Fat content measured after hydrolysis.	Suitable
By-products from maize	High fat- and linoleic acid contents: maizeglutenmeal and maizeglutenfeed *(7)*. Fat content measured after hydrolysis.	Not suitable

Table 6
By-Products From Sugar Plants

Cane molasses	High levels provoke wet droppings.	Suitable Max 4%
Sugarbeet pulp		Not suitable

Table 7
By-Products From Distilleries and Breweries

Brewer's yeast	Quality (total crude protein and amino acid content) highly fluctuates depending on the origin.	Suitable Low level

Table 8
Dried Bulbs, Tubers, and Roots

Tapioca	Very low fat content. Considered as a substitute for cereals based on its high content of starch (60–70%). Is being commercialized in the form of chips or pellets. Because of large variation in mesh structure, use in layer feed is rather limited.	Suitable
Sweet potatoes, dehydrated	Low fat content. Can be used in limited amounts, such as manioc. Are available in the form of chips.	Suitable Low level

Table 9
Oleaginous Seeds

Chiaseeds (*Salina hispanica* L.)	Considering the very small size of the seeds—they can partly be traced back in the manure—further study on the use of grounded chia seeds in layer feed might prove interesting. Chia seeds are a raw material with great potential for future use in the layer sector and in ω-3 egg production. When used in layer feed up to a level of 28%, the eggs show no taste abnormalities *(8)*.	Suitable Max 28%
Flax seed	Flax seeds are rich in energy and protein. The fat fraction contains much ALA and produces flax seed oil after extraction. Flax seeds are the only oleaginous seeds containing slime (mucilage), between 3 and 10%. Nonruminants cannot digest mucilage and, added to water, it forms a gel. Mucilage works as a laxative. Flax seeds contain	Suitable Max 10%

(Continued)

Table 9 *(Continued)*

	linamarine, a cyano-derivatized glucoside. The in-seed built myrosinase enzyme releases HCN under humid conditions.	
	According to Eder et al. *(9)* the level of flax seeds in layer feed should not exceed 10%. Level of 15% adversely affects feed conversion and egg weight *(10)*, probably resulting from the antioestrogenic effect (reduction of the amount of circulating oestradiol responsible for the lipogenese in the liver and the yolk formation in the ovary) of high loads of ALA and phyto-oestrogens present in flax seeds *(11,12)*. According to Eder et al. *(9)* the level of ALA deposited into the yolk is 10 to 20% higher with grounded than with whole flax seeds. Linseed can be mixed with cereals and consequently grounded in a hammer mill. The deposition of ALA in the yolk has been reported to be higher with older than with younger hens *(10)*. Different strains show only minor differences in the fatty acid profile of the yolk as a consequence of different feed treatments.	
	ALA leads to the formation of DHA and, to a smaller extent, to the formation of EPA (*see* Fig. 1). LA leads to the formation of ARA. As both metabolic pathways use the same enzymes, there is competition between them. ALA is not an essential nutrient and is easily deposited into the yolk. The ALA content in the yolk increases in a linear fashion with the increasing contribution of flax seed in the feed. The metabolic derivatives of ALA (i.e., DHA and EPA), are only deposited in a limited way into the yolk, regardless the quantity and the form of flax seeds *(13)*.	
Rapeseed	Through natural selection, a 00 (double 0) variety with a low erucic acid content (less than 2%) and a guaranteed maximum level of glycosinolates (less than 20 μmol/g) has been isolated. However, this 00-variety presents a number of additional problems for use in poultry: the fiber content in the seed remains high and affects digestibility; the percentage of sinapin remains high and this substance is held responsible for the accidental development of abnormal fishy taste, resulting from the production of trimethylamine (TMA) in the intestine. In principle, TMA is oxidized—and therefore deodorized—upon resorption. However, brown-egg layer strains do not have this oxidative process.	Not suitable
Sunflower seed	High fat and linoleic acid contents.	Not suitable
Soyabeans heat-treated	High fat and linoleic acid contents.	Not suitable

Table 10
By-Products From the Vegetable Oil Industry

Soyabean, extracted	Low fat content.	Suitable
Sunflower seed, extracted	Low fat content. However, less interesting because of low energy and low lysine- and threonine values.	Suitable Low level
Rapeseed, extracted	Not indicated for similar reason as for rapeseed (cfr. VIII).	Not suitable
Flax seed expeller	The low digestibility of the protein (56% *[w/w]*) and the other carbohydrates (22% *[w/w]*) make this raw material practically unsuitable for layer feed because of its low energy content.	Not suitable

Table 11
By-Products of Animal Origins

Marine algae	Good source of ω-3 LC-PUFA. Dried fermented products contain ±7% DHA in a natural triglyceride form. The ω-3 LC-PUFAs are more stable and available in algae than in fish oil because of their dispersion. Layer feed containing 4.8% marine-algae results in the deposition of approximately 180 mg total ω-3 LC-PUFAs—mainly DHA—into the yolk *(14)*.	Suitable Max 5.0% for ω-3-enriched Columbus eggs
Fishmeal	Fatty fishmeal is rich in ω-3 LC-PUFAs. According to Van Elswijk *(15)*, the ω-3 fat content of the yolk reaches, in a dose/response trial, a peak (150 mg ω-3 LC-PUFA/yolk) at 1.5% menhaden fish oil in the ration. Higher values cause a dominant fishy-taste in boiled eggs. Thus fishmeal is a raw material suitable for the production of ω-3-enriched eggs, however with some reserve with regards to development of fishy flavor.	Suitable Max 1.5% for ω-3-enriched Columbus eggs
Animal meal	At present not allowed in Europe, on account of the BSE problems. Yet is its current fatty acid pattern not suitable for the production of ω-3 eggs.	Not suitable

Table 12
Oils and Fats

Fish oil	The EPA/DHA in the yolk seldom exceeds 0.15 when layer mash feed contains (menhaden) fish oil. According to some scientists (menhaden) fish oil has no influence on the technical results *(16)* whereas others claim that menhaden fish oil has a negative effect on production parameters, especially on egg weight *(17)*. The biggest problem with fish oil is the	Suitable for ω-3-enriched Columbus eggs

(Continued)

Table 12 *(Continued)*

	development of a "fishy smell". This is related to the higher sensitivity to oxidation resulting from high levels of unsaturated ω-3 fatty acids. Such problems seem to be overcome with the supplementation of synthetic antioxidants and vitamin E.	
Algae oil	Algae ω-3 fatty acids are derived from artificially grown algae (Omega Tech-products). In trials with DHA gold™ *(14)*, the incorporation of 24 of 48 g/kg feed of this additive results in the introduction of between 10 and 12 mg of ω-3 LC-PUFAs/g yolk. These results show that 24 g algae product is equivalent to 15 g menhaden fish oil. Although the results with algae ω-3 fatty acids are promising, the cost price of the algae is rather high and has a great economic impact on the production of ω-3 eggs.	Suitable for ω-3-enriched Columbus eggs
Flax seed oil	Bancells et al. *(18)* state that the substitution of fish oil for flax seed oil does not significantly influence production parameters. The substitution gradually decreases the DHA content from 10.6 mg/g yolk to 5.2 mg/g yolk whereas, in the same time, the ALA content increases from 1.5 mg/g yolk to 16.3 mg/g yolk, and the ARA content increases slightly from 2 mg/g yolk to 3 mg/g yolk. These results again demonstrate that the presence of the ω-3 fatty acids (ALA, EPA, and DHA) in the feed decreases the activity of the Δ6-desaturase and that EPA and DHA are more efficient to this respect than ALA.	Suitable
Other oils and fats	See *(19)* for a review of the influence of 9 different oils on the yolk-structure and production parameters.	Not suitable

Table 13
Dehydrated Grassmeal

Alfalfa	Source of protein and natural color (lutein).	Suitable

3. CONCLUSION

The production of Columbus layer feed requires selecting raw materials that impart maximum input of ω-3 fatty acids and minimum input of ω-6 fatty acids. Doing so, one obtains the optimal ratio's ω-6:ω-3 = P:S = 1:1. The deposition of ω-3 LC-PUFA in the yolk is not linear with the increasing amount of ALA in the ration and shows a level of saturation. Higher levels of ω-3 LC-PUFAs in the yolk may cause the development of an abnormal smell and taste and requires additional precaution with regards to the level of antioxidants.

Feeding trials are the only conclusive way to find out if a ω-3-enriched feed formulation for the design of a second generation of ω-3-enriched Columbus eggs meets the requirements in terms of production yield, sensorial characteristics and market penetration.

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38 Modification of a National Diet and Lifestyle Toward Wild-Type Foods

The Cuban Experience in Promoting Health

Alfredo Nasiff-Hadad
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Abstract

Cuba's economy has undergone serious constraints since the begininng of the 1990s. A comprehensive analysis of food availability, food consumption, nutritional status, physical inactivity, smoking habit, and mortality rate for cardiovascular disesase and stroke from 1988 to 2004 was carried out and the main issues concerning lifestyle patterns were identified. On the basis of this information, the main results obtained reveals a reduction in consumption of total energy, total amounts of fats, cholesterol, animal protein, dairy products, and increased consumption of vegetable, tubers, cereals, soja bean products, and beans, as well as decreased prevalence of obesity among people older than 15 yr age, less sedentary people, and reduction in the prevalence of smokers in the 1990s. A concomitant reduction on cardiovascular mortality rate was observed at the same time. Even though the hypothesis supported the positive impact of the economic crisis of the early 1990s on the public health epidemiological profile there is not evidence confirmed based on epidemiological studies. It will be necessary to follow-up on those parameters in the coming years.

Key Words: Health promotion; lifestyle, diet; noncommnunicable disease; risk factors.

1. INTRODUCTION

In order to prevent cardiovascular diseases (CVD) and to treat those patients that already suffer them it is necessary to take into consideration strategies that are aimed at promoting healthy diets and lifestyles affecting all sectors of society, not just the health sector *(1,2).*

Some studies have demonstrated that the elimination of tobacco use *(3,4),* habitual physical exercise *(5–7),* the loss of weight in obese and overweight *(8,9),* and the incorporation of healthy nutritional habits can contribute, to reduce mortality for, cardiovascular events.

Unfortunately, not all the people at risk for CVD risk fulfill the proposed recommendations appropriately to reach a lifestyle that reduces the risk *(10)*. The changes of the lifestyle demand self-discipline of those who seek to incorporate new attitudes or to abandon long-practiced customs *(11*).

To ask the whole population of a country to simultaneous change their lifestyle would be quite difficult. It is of scientific interest to expose changes in lifestyle habits

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the population of the Republic of Cuba during the 1990s, as it resulted from a severe economic crisis and could be favorably related to changes in CVD mortality rates observed during this period.

2. EPIDEMIOLOGICAL AND NUTRITIONAL TRANSITION

The health situation in Cuba has undergone much transformation, resulting mainly from demographic and socioeconomic changes and from rapid urbanization. The fecundity rate in Cuba is under the level of replacement (i.e., less than one girl child/woman) and the population beween 0 and 14 yr of age has decreased with relation to the total population; at the same time there has also been a decrease in the mortality ratio among elderly people (i.e., > 60 yr of age) *(12)*. The decrease in the mortality and fecundity ratios and the increase in the life expectancy (population above the age of 60 is growing at a rapid rate <15.4% of the total) *(13)* have produced a change in the shape of the population pyramid. On the other hand, noncommunicable diseases have became more prevalent than communicable diseases.

Epidemiological transition comprises long-term modifications in the mortality, disease, and discapability profiles in a given population *(14)*. It has been shown that these modifications are related to demographic, socioeconomic, and food changes. In Cuba those changes occurred very fast, as in Chile and Costa Rica. This process started at the end of the 1950s in Cuba, together with a rapid and sustainable decrease in the prevalence and incidence of infectious diseases. CVD and cancer were the primary causes of death at that time *(15)*. Social changes and the implementation of a general health policy from 1959 produced many positive changes in the health situation and had a very important impact on the epidemiological transition.

The Nutritional Transition describes changes in the food pattern, physical activity level, health, and nutritional status resulting from changes in the food structure of the people as a result of their socioeconomic, sanitary, and demographic changes.

Nutritional Transition is expressed in terms of food pattern and nutritional requirement changes of a given population and the presence of acute undernutrition, growth retardation, obesity, noncommunicable diseases, and specific micronutrients deficiency. Diet is usually related to the morbidity and mortality patterns of different communities and countries *(16)*. In Cuba, CVD is the most frequent cause of death, and the trend indicates this will continue to be a major problem unless the risk factors are controlled. Cancer and cerebrovascular diseases are the second- and third-highest causes of death, respectively. Obesity tends to increase among children and adults; conversely, undernutrition no longer presents as a public health problem. There has also been an increase in the prevalence of obesity among childrens and adults as 75.8% of the Cuban population is already living in urban areas *(13)*. This means greater female participation in the labor force and a movement toward fast food and convenience food at home. Urbanization also implies less physical activity and more sedentary lifestyle.

To be able to understand this transitional process it is necessary to focus on adequate on-time food aid programmers and to train the new generations of professionals to reach the new society demands.

3. STUDY METHODS

3.1. Food Consumption Assessment

Assessment of the dietary shifts on lifestyle and the impact on cardiovascular and cerebrovascular diseases is described. The epidemiological profile since 1998 is also described in relation to the apparent consumption of foods and macronutrients. These data were obtained from the National Statistics Office. Food consumption is measured from a food supply perspective. Information is obtained from a basket of 95 food comodities distributed through different manners: subsidized foods, free-market foods, vulnerable groups, small farms and cooperatives, and the household production of foods. Software was used to evaluate the macro- and micronutrient content of all foods. Loss and waste of nutrients resulting from distribution and processing was also considered. Therefore, through this methology the data obtained is believed to be accurate and representative of the actual nutrient consumption of the population. Because the basic data are obtained from different sources, they are subject to a quality control in order to avoid inconsistency.

Each food commodity figure was obtained by dividing the food apparent consumption figure by the total population during the reference period. In this calculation, food consumption from special groups such as tourists and foreign diplomatic personnel and their dependents are not included.

3.2. Evaluation of Chronic Disease Risk Factor

Data from the two national surveys on risk factors and noncommunicable diseases carried out by the National Institute of Hygiene and Epidemiology and the National Statistics Office and supported by the Institute of Nutrition and Food Hygiene are included in this chapter. The first study was carried out between February and April 1995 and included 14,304 individuals, whereas the second study, carried out between November 2000 and March 2001, included 22,851 individuals. Both studies are representative samples of the Cuban urban population older than 15 yr. The purpose of these surveys was to identify and describe the key noncomunicable diseases risk factors *(17)*. Height and weight were measured in duplicate using weighing scales periodically calibrated and steel measuring tapes. Body mass index (BMI) was calculated as weight (kg)/h (m^2). The cut-off point used for BMI is shown in Table 1. Smoking prevalence was estimated on the basis of expert criteria. For the purpose of the study, a smoker was defined as an individual who was smoking at the time of the study or has been regularly consuming tobacco for the previous month. The intensity of a population's habitual physical activity and leisure activity was classified according to the World Health Organization (WHO) *(18)*.

3.3. Mortality and Morbidity Information

Data on mortality were obtained from the National Satatistic Department of the Ministry of Public Health annual reports. Information on mortality from malignant tumors was obtained from the National Cancer Register. Mortality rates were age-standardized with reference to the population of 1981 *(19)*.

Table 1
Cut-Off Points for Body Mass Index

Cuttof point	*Risk group*
<16.0	CED-III
16.0–16.9	CED-II
17.0–18.4	CED-I
18.5–24.9	Normal
25.0–29.9	Overweight-I
30.0–39.9	Overweight -II
≥40.0	Overweight-III

Abbr: CED, Chronic energy deficiency.

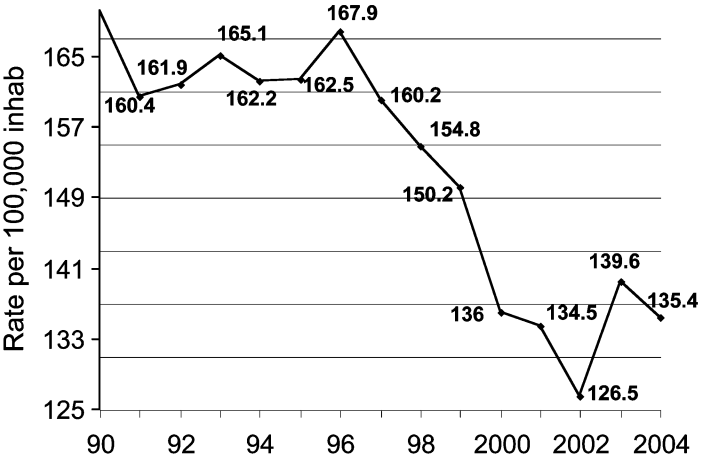

Fig. 1. Mortality rate from ischemic heart disease. All ages. Cuba 1990–2004.

4. INDICATORS OF MORTALITY FOR CVD AND MORBIDITY FOR CANCER IN CUBA

Cuba is a developing country. However, the infectious and parasitic diseases are not primary causes of death. The national system of healthcare indicates mortality rates similar to those of developed countries. In 2004, the total number of deaths was of 81,103. The three leading causes of death were heart disease (20,995 defunctions), malignant tumors (18,715 defunctions), and cerebrovascular disease (8268 defunctions). These primary three causes account for 59% of all deaths.

The tendency of the mortality for ischemic heart disease and stroke during the 1980s stayed practically the same. However, throughout the 1990s, a decreasing trend of the mortality for the three leading causes of death was observed (see Figs. 1 and 2). This tendency remained until the year 2002, when it began to increase again. The mortality rate for ischemic heart disease diminished from 167.5 in 1996 to 126.5 in 2002.

The morbidity for tumors did not exibit the same tendency as ischemic heart disease in the same period. Comparing data on men from 1988–1990 with that of 2002, there was a

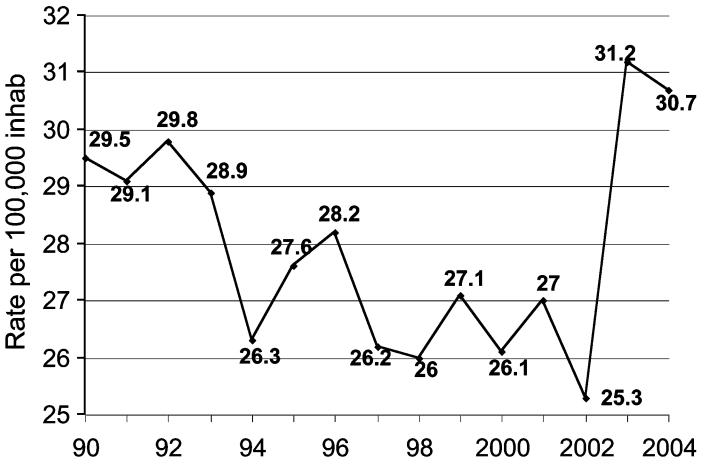

Fig. 2. Mortality rate from stroke. All ages. Cuba 1994–2002.

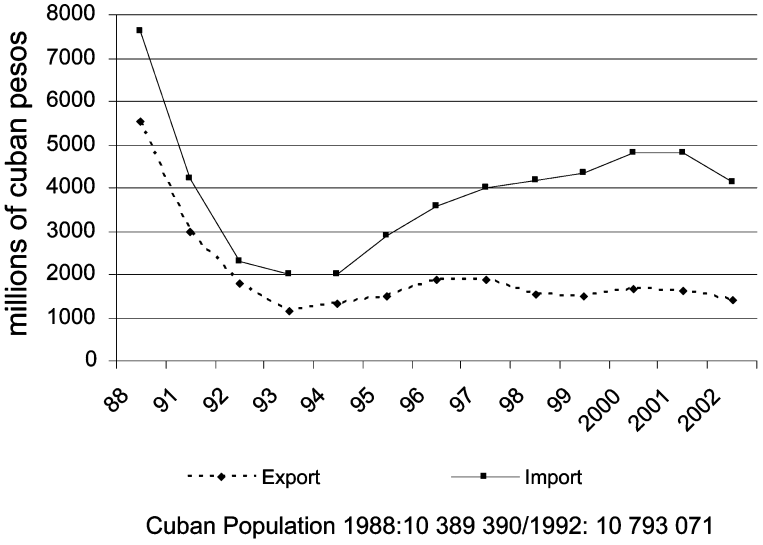

Fig. 3. Importation and exportation. Cuba 1988, 1991–2002.

slight increase in the most common types of tumors (e.g., lung, prostate, and colon), whereas in women the increase was higher (i.e., breast cancer increased from 33.2 to 39.5%, lung cancer from 18.4 to 24.1%, and colon cancer from 13.1 at 18.8%).

The 1990s marked a drastic reduction in Cuban imports and exports (*see* Fig. 3), the negative sanitary consequences of which were revealed in 1993 with the Cuban neuropathy epidemic—an epidemic characterized by a sensitive-motor bilateral neuropathy that predominantly affected the inferior limbs, as well as bilateral optic neuritis. Etiology showed a nutritional and toxicological origin.

The distribution of foods is very homogeneous. Furthermore, social feeding (e.g., day-care centers, schools, and workplaces) covers more than 30% of the recommended dietary intake of energy and nutrients and is subsidized by the state. On the other

Table 2
National Average Apparent Consumption of Energy Derived From Any Source and Percentage of This Energy Derived From Carbohydrates, Fats, and Proteins

Year	*Total energy (kcal)*	*Total carbohydrates (from sugar) (%)*	*Fats (%)*	*Proteins (%)*
1988	2908	66 (19)	20.30	10.71
1992	2183	76 (29)	16.23	9.22
1993	1863	78 (30)	12.40	9.81
1994	1948	77 (29)	13.29	9.80
1995	1993	76 (28)	13.59	9.97
1996	2160	78 (28)	11.98	9.74
1997	2193	77 (27)	12.94	9.98
1998	2244	74 (23)	15.35	10.97
1999	2429	76 (25)	13.26	10.68
2000	2570	78 (24)	11.42	10.20
2001	2758	78 (23)	12.36	10.09
2002	2785	76 (21)	14.39	10.11
2003	2936	77 (23)	12.91	9.94
2004	3226	77 (19)	12.83	10.27

Source: Food, Hygiene and Nutrition Institute.

hand, the access to health services is free of charge for the whole population, transportation costs are low, and most of the people use collective transports.

Taking into consideration the different factors mentioned above we can formulate the next question: Which factors influenced the reduction of ischemic heart disease mortality rate in the 1990s in Cuba, in spite of the affectations suffered in health sector, resulting from the lack of production and commercialization of medications and reagents for diagnostics, as well as the limitations in some resources used in surgery, cardiology and other specialties?

Atherosclerosis is the cause of ischemic heart disease and stroke. Cardiovascular risk factors are responsible for the development of atherosclerosis. Changes in lifestyle are necessary to control these risk factors.

5. FOOD CONSUMPTION

Average supply figures for energy and the percentage of energy derived from carbohydrates is presented in Table 2. Apparent consumption of energy/day from 1988 to 1993–1994 in the adult population decreased dramatically by more than 1000 kcal/capita/d. These changes resulted from the adaptation to a new economy marked by reduced trade and tightened controls.

Those changes affected the availability of resources for production and consumption. These food, production, and import shortages resulted in a sharp decrease in the overall food supply within the country, resulting in inadequate energy and nutrient intake (Table 3).

Table 3
Daily Per Capita Apparent Consumption of Energy From Fats and Total Amount of Cholesterol

Year	*Total %*	*Saturated %*	*Monounsaturated %*	*Polyunsaturated %*	*P/S ratio*	*Cholesterol (mg)*
1988	23.30	6.30	10.2	6.33	1.0	513
1992	16.23	4.90	6.85	3.48	0.7	286
1993	12.40	4.12	5.69	2.59	0.6	209
1994	13.29	4.42	6.10	2.78	0.6	189
1995	13.59	4.76	6.44	2.39	0.5	178
1996	11.98	3.78	5.00	2.60	0.7	199
1997	12.94	4.14	5.98	2.83	0.7	209
1998	15.35	5.12	7.17	3.06	0.6	241
1999	13.26	4.16	6.16	2.96	0.7	232
2000	11.42	3.78	5.79	2.80	0.7	256
2001	12.36	3.78	5.79	2.80	0.7	221
2002	14.39	4.13	6.24	4.03	1.0	257
2003	12.91	3.87	5.49	3.54	0.9	241
2004	12.83	3.25	4.51	3.59	1.1	282

Source: Food, Hygiene and Nutrition Institute.

In order to overcome that situation, a national policy based on the following aspects was launched:

- Diversification of foreign markets and exports.
- Savings in all sectors, especially in energy expenses.
- Orientation toward reasonable food self-sufficiency.
- Strengthening of traditional export goods, among others.
- A more prominent role of the individual population as active players in the National Food and Nutrition Program, promoting self-sufficiency throughout the country, and improving the dietary cultural patterns *(20)*.

Since 1996, important and sustainable energy supplies have been obtained and in 2004 the Cuban recommended dietary intake for energy (2400 kcal) figures were much higher than those of 1988, with adequacy levels reaching 134.

Relative contribution of energy from carbohydrates increased remarkably from 1988 to 1993 and sugar contribution to energy supply is very high (Table 2).

Apparent consumption per capita of total protein also decreased in the same period mentioned above. However, the relationship between animal protein and vegetable protein changed favorably as result of an increase in consumption of protein from vegetable origin.

During the 1990s, the main alteration in the Cuban diet was a decrease in the amount of dietary fats (Table 3). In recent years, the percentage of vegetable fats has been increasing. The rate of energy from saturated, monounsaturated, and polyunsaturated fats is currently within the limits of healthy international parameters *(21)*. The consumption of cholesterol decreased significantly.

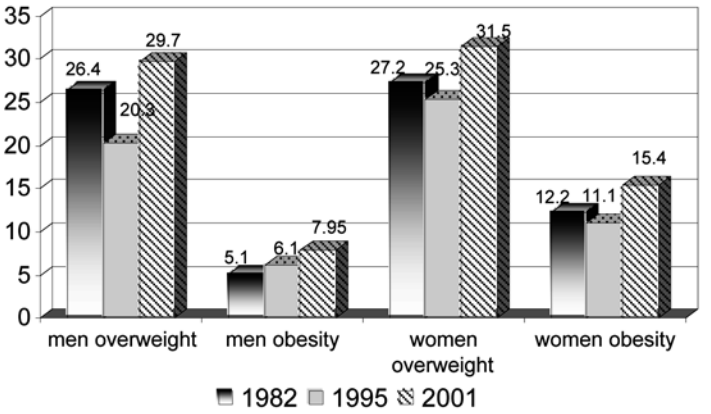

Fig. 4. Overweight and obesity prevalence in Cuban adults in three periods.

6. MAIN TRENDS IN FOOD CONSUMPTION DURING THE 1990s

With regard to apparent consumption of foods for the whole population, three tendencies were evident between 1988 and 2004:

- Minor changes occurred concerning foods that are main sources of energy, such as cereals.
- A decline was observed after 1990 in the supply of animal protein from milk, eggs, fish, and meat, contrasting with the increasing trend before this period.
- Intake of vegetables, starchy roots, and tubers, plantain, and soy bean products increased. It is important to note that sugar accounts for nearly 25% of the total energy value of average diet.

Looking for alternatives to improve family food security, the government decided to deliver plots of land up to 0.2 ha/family for food production, intended to create self-supplying households that could then sell surplus foods at tax-free prices *(22)*. Farmers' markets, created in the mid-1990s, offered the option to buy certain foods tax free, yet still at high prices—depending on supply and demand—so that a broader range of food products were available for consumption. A strategy to recover agriculture in mountainous places was launched in order to encourage suitable and sustainable crops.

In the study of the Cuban adult population, obesity values from 1995 (First National Survey of Risk Factors) (*see* Fig. 4) were lower than in 1982 *(23)*. The prevalence of obesity in both surveys was higher in females than males. The Second National Survey of Risk Factor, carried out in 2001, showed an increasing trend of obesity and that the 1995 reduction did not result from conscientous changes in lifestyle. Prevalent food habits and attitudes among Cubans are deeply rooted in the process of cultural transfer from Spain and Africa, which began almost five centuries ago and dates back to colonial times. The lack of a previously developed culture in Cuba, the attempts of inmigrants to adapt themselves to an unknown environment, and various socioeconomic struggles have influenced the living conditions, behavior, and particularly the food habits of the country.

At the present time, the dietary patterns, of the Cuban population cannot be regarded as nutritionally adequate. The populations knowledge of nutritional value and proper food preparation techniques are still inadequate. Therefore, a multisectorial Campaign

for the Improvement of Food and Nutritional Culture has been implemented throughout the country, aiming to raise the level of knowledge and consciousness of food croppers, manufacturers, grocers, caterers, cooks, and consumers on the principles and practices of a rational diet.

7. CONCLUSIONS

There is no doubt that the health status of the Cuban population, is at present, far from being characteristic of a developing country. Conversely, in many aspects it has attained a pattern close to that of the industrialized world. Much of this results from the high priority given to the social programs, namely to public health, and to the active involvement of the community.

The outcome of the unexpected reduction of essential nutrients and energy associated with a sudden increase in physical activity and the reduction of the mortality rate from ischemic heart disease and stroke mortality cannot be considered a "healthy" intervention program.

Another aspect to take into consideration is the national program to reduce smoking, which includes increasing the price of cigars and cigarettes, as well as prohibiting smoking in closed areas, health centers, and schools.

The increased availability of food in the last few years and the observed reduction of physical activity (less people cycling and walking), coupled with an increase in obesity, could be the underlying cause of the current trend in CVD. Even though this hypothesis supported the positive impact of the economic crisis of the early 1990s on the public health epidemiological profile, evidence has not been confirmed based on epidemiological studies. Much remains to be done in order to improve the nutritional status of the population.

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39 Studies on the Effects of Diets Realistic for Westernized People on Plasma Lipoprotein Composition, Metabolism, and Atherosclerosis in Vervet Monkeys

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Abstract

Using diets realistic for Westernized people, which consist entirely of normal foods for humans, has added to our knowledge regarding the impact of diet on plasma lipoprotein metabolism and atherosclerosis in Vervet monkeys. The overall effect of a Western atherogenic diet (WAD) on the progression and a prudent diet (PD) on the regression of atherosclerosis were confirmed. Although atherosclerosis is closely associated with increased levels of low-density lipoprotein cholesterol (LDL)-C, the increased levels of LDL-C measured in these studies were brought about by an increased number of circulating LDL particles of relatively unchanged composition without enrichment of the LDL core with cholesterol esters.

This model proved to be valuable for studying the effects of a variety of dietary supplements such as fish oil, EPA, palm olein and blood lipid lowering agents such as etofibrate, on plasma lipoprotein metabolism, LDL composition, LDL turnover and atherosclerosis. Results from the studies with eicosapentaenoic acid (EPA) and gammalinolenic acid (GLA) supplementation showed a slower rate of metabolism of these fatty acids in animals consuming the WAD. These findings signal a warning against the indiscriminate use of supplements by individuals at risk of hyperlipoproteinemia. Results from the studies following the disappearance of EPA and GLA from tissues after supplementation provide a potential model for determining the daily requirement of n-3 fatty acids in an individual under different dietary conditions.

Key Words: Realistic diets; lipoprotein metabolism; atherosclerosis; Vervet monkeys.

1. INTRODUCTION

Numerous studies carried out during the last three decades have shown that some species of nonhuman primates are good models for both the type and composition of atherosclerotic lesions developed, and the changes in plasma lipoproteins found in response to diets, as occurs in man *(1–8)*.

Direct comparison of results that emerged from these studies are complicated by potential species differences and their responses to dietary intervention as well as by the different dietary approaches used during the intervention. Diets used in several of these

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studies were often loaded with up to 2% cholesterol and with fat from sources such as butter, coconut, or sunflower oils *(9–15)*. Such loading was applied in order to accelerate atherogenesis and obtain quick results, but it deviates from realistic nutrition and the time scale of atherogenesis. In man, atherosclerosis can progress for more than 50 yr before the disease is clinically detectable *(16)*. These discrepancies may invalidate many research results on comparative atherogenesis.

Against this background, researchers at the Medical Research Council of South Africa formulated realistic diets for Westernized people consisting entirely of normal foods for man in orderto investigate the effects of diet on lipoprotein metabolism and atherosclerosis in Vervet monkeys. Extensive research based on this model was carried out by this group of researchers during the last two decades. Results from these studies form the basis for this review.

2. THE COMPOSITION OF DIETS REALISTIC FOR WESTERNIZED PEOPLE

To obtain results relevant to diet and disease in man, dietary treatments were formulated entirely from normal foods for man *(17)*. These treatments reflected dietary habits of Westernized people over a range from dietary prudence advocated by bodies like the American Heart Association (AHA) to over-indulgence associated with affluence and ignorance. Three different diets were initially formulated namely, a high-carbohydrate diet (HCD), a prudent diet (PD), and a western diet (WD). The percentage of energy derived from fat was 12, 29, and 47% and from carbohydrate 75, 57, and 39%, respectively, for the HCD, PD, and WD. Protein supplied 14% for all 3 diets. Cholesterol content for the diets was as follows: HCD traces, PD 0.08 mg/kcal and 0.33 mg/kcal for the WD. The polyunsaturated to saturated fat ratios were 1.94, 1.77, and 0.3, respectively for the HCD, PD and WD.

Batches of raw ingredients were minced, mixed, shaped into patties, and baked at 180°C for 30 min. Stocks of baked patties were stored in a domestic freezer (–20°C) for up to 3 wk before feeding. The feeding routine was to distribute the patties twice/d at set times, thereby reproducing major feeding behavior of adult Western man. No pure cholesterol was added to the diet to accentuate the cholesterolaemic response.

3. THE EFFECT OF DIETS FOR WESTERNIZED PEOPLE ON LIPOPROTEINS AND ATHEROSCLEROSIS IN VERVET MONKEYS

Fincham et al. *(1,17)* described measurements of 50 variables in adult female, reproductively inactive Vervet monkeys during prolonged nutrition realistic for Westernized people. Randomized groups of Vervet monkeys received either the PD or WD for 47 mo, whereas a third group was fed the WD for 20 mo and then the PD for 27 mos (WD-PD). Before the dietary treatments commenced Vervets received a HCD, values of which were used as baseline and reference values.

Plasma total cholesterol was increased from 147 mg/dL (HCD) to 174 mg/dL (PD), and 376 mg/dL (WD). Individual cholesterolaemic response ranged from mild to severe. Dietary reversal (WD-PD) reduced cholesterolemia promptly. The WD was found to be atherogenic in aortas and some arteries. The PD induced much less lipid

infiltration into aortic intimas and was not associated with serious atherosclerotic changes. When this PD was used as a treatment for 27 mo, following 20 mo of nutrition by the WD, minimal regression of cholesterol crystals and nonlipid components of atherosclerotic plaque were detected. The authors concluded that for this model the PD would be more effective in preventing atherosclerosis than treating advanced lesions. This was also the first time that the pathology of atherosclerosis promoted by realistic diets has been modeled in adult females of this species.

4. LOW-DENSITY LIPOPROTEIN KINETICS IN VERVET MONKEYS CONSUMING DIETS REALISTIC FOR WESTERNIZED PEOPLE

Weight et al. *(18)* studied the effect of a WD, PD, or the HCD for various lengths of time, on LDL kinetics as determined by native^{125}I-labeled LDL and reductively methylated^{131}I-labeled LDL. Monkeys differ greatly in their response to the WD, and a strong negative correlation was observed between the fractional catabolic rates (FCR) of LDL and the total plasma cholesterol concentrations ($r = –0.83$; $p = 0.0029$). Individual synthetic rates (SR) of LDL showed a significant correlation with plasma cholesterol for WD animals ($r = 0.94$; $p = 0.01$). No correlation was found for PD or HCD for either SR or FCR and total plasma cholesterol concentrations. Aortic lesion scores correlated with the SR's ($r = 0.66$; $p = 0.040$) but not with FCR's.

LDL turnover was also studied using ^{125}I-labeled native LDL (measuring total clearance) and methylated ^{131}I-labelled LDL (measuring non-receptor clearance) in Vervets showing a high or a low cholesterolemic response to the WD. Measurements after 4 wk and again after 6 mo on the diet showed a decrease in both total and nonreceptor mediated clearance of LDL. Receptor-mediated catabolism was significantly lowered in the hyper-responding group. These authors also reported a repression in the clearance of methylated LDL after feeding the WD to hypo- and hyper-responding Vervets for 6 mo. However, the hyper-responding group was depressed to a significantly greater extent when compared with the hypo-responding group ($p = 0.0241$).

These results strongly suggest that part of the explanation for the hyper-cholesterolaemic response observed when Vervets are fed a WD, is a lower FCR, and increased SR of LDL—apo B, probably resulting at least in part from the increased absorption of cholesterol from the intestine *(9)*. Vulnerable hyper-responding individuals respond with hyper-cholesterolemia and accelerated atherosclerosis *(1)*.

5. PLASMA LDL COMPOSITION IN RELATION TO ATHEROSCLEROSIS IN NUTRITIONALLY DEFINED VERVET MONKEYS

Benadé et al. *(19)* investigated the effect of an atherogenic diet (WD) on the LDL composition in Vervet monkeys. Diets consisted entirely of normal foods for Westernized people, which was fed for 4 yr, Plasma LDL cholesterol pool was increased and progression of atherosclerosis was enhanced by the WD compared with a more prudent Western diet. The increased LDL cholesterol was carried by a threefold increase in particles of relatively normal composition and not by packing cholesterol esters into the cores of

enlarged LDL particles, as has been reported after feeding semisynthetic diets loaded with extra cholesterol *(9,13)*. Nevertheless these LDL particles were atherogenic.

Fatty acid profiles of subcutaneous fat between the diets reflected the fatty acid profiles of the respective diets, namely higher C 16:0 (palmitic acid), C 18:0 (stearic acid), C 18:1 (oleic acid) and lower C 18:2 (linoleic acid) in the Western diet than in the prudent diet. A similar tendency was observed in the fatty acid composition of LDL-cholesterol esters.

Multivariate analysis showed that measures and scores of atherosclerosis were significantly dependant on sphingomyelin (SM) and phosphatidylcholine (PC) in LDL and on arachadonic acid (C 20:4) in LDL triacylglycerol (TAG). Although apolipoprotein B, free cholesterol (C), esterified cholesterol (CE), and lysophosphatidylcholine (LPC) in plasma LDL and atherosclerosis were significantly positively correlated in bivariate analysis they were not selected by multivariate analysis as the strongest determinants of atherogenesis. Cholesterol in plasma high density lipoprotein (HDL-C) was not changed by the WD. Lecithin-cholesterol acyltransferase (LCAT) activity in plasma was inversely linked to atherosclerosis.

Subcutaneous fatty acid reflected dietary fatty acids. The correlations found between fatty acids in subcutaneous fat and arterial lesions suggest that analysis of superficial fat could be of practical value in assessment of atherosclerosis risk.

6. THE EFFECT OF THE AMOUNT AND DEGREE OF UNSATURATION OF DIETARY FAT ON PLASMA LDL IN VERVET MONKEYS

Kruger et al. *(20)* studied the effects of the degree of unsaturation and of the amount of dietary fat on plasma LDL concentration and composition in Vervet monkeys. Diets with fat contents of 41, 31, and 18% energy, each with a low and a high polyunsaturated to saturated fatty acid ratio (P:S 0.27–0.38 and 1.13–1.47) were fed to 6 female Vervet monkeys for 2 mo. Cholesterol content of the diets were low (21–33 mg/d) and relatively constant. LDL-cholesterol concentrations decreased significantly ($p \leq 0.01$) when the dietary fat content decreased from 31 to 18% of energy. The dietary P:S ratio only affected LDL-cholesterol concentrations during moderate (31% of energy) fat intake, where LDL-cholesterol increased ($p \leq 0.01$) with a decrease in dietary P:S).

Substantial individual variations were observed in LDL-cholesterol concentration responses to dietary fat changes. The changes measured in LDL-cholesterol concentrations were the result of changes in the concentration of LDL particles, as the molecular composition did not differ significantly between dietary periods. Plasma triacylglycerol (TAG) and HDL-C were not influenced by the dietary fat changes. During the high P:S diets, the percentage of C 18:2 (linoleic acid) increased significantly ($p \leq 0.01$) and that of C 18:1 (oleic acid) decreased significantly ($p \leq 0.01$) in the LDL esterified cholesterol, as compared with the low P:S diets.

In adipose tissue TAG, the percentage of C 18:2 was three times higher ($p \leq 0.01$) during the high P:S diets than during the low P:S diets.

The strong effect of the P:S of the diet on the fatty acid composition of lipoprotein and adipose tissue lipids agreed with previously reported results in humans *(21)*.

7. INFLUENCE OF FISH OIL SUPPLEMENTATION ON PLASMA LIPOPROTEINS, ARTERIAL LIPIDS, AND ATHEROSCLEROSIS IN VERVETS

Smuts et al. *(22)* and Fincham et al. *(23)* investigated the effects of Atlantic pilchard *(Sardinops oscellata)* oil supplement on atherosclerosis in Vervet monkeys fed a WAD diet. Atherosclerosis was induced by feeding Vervets a WAD for at least 15 mo. Matched groups were then treated for 20 mo, either by adding the fish oil (FO) to the WAD (WAD-FO), or by changing to a therapeutic diet with FO (TD-FO). Control treatments consisted of supplementing with sunflower oil (SO) instead of FO. Polyunsaturates supplied by the FO and the SO was equal and the FO supplied 2.5% of total energy.

Supplementing with FO did not change the concentrations of total cholesterol, LDL-C or HDL-C in plasma. Aortas from the WAD-FO group contained significantly more total, free and esterified cholesterol, total phospholipids, and sphingomyelin than after the WAD-SO. Fish oil supplementation increased eicosapentaenoic acid (C 20:5) significantly in plasma and aorta intima phosphatidylcholine. Arachadonic acid (C 20:4) was significantly lower in these tissues.

No component of atherosclerosis regressed after dietary FO, and several deteriorated. Change to the TD cleared stainable lipid from aortas. Advanced atherosclerosis was not reduced. Fish oil did not increase the efficacy of the TD.

These two studies differed from other n-3 studies involving primates in so far that a realistic FO dose (2.5% of energy) was used and that the effects of n-3 polyunsaturated fatty acids were measured against an equivalent dose of n-6 polyunsaturated fatty acids (SO). This was in contrast with other experiments with nonhuman primates in which FO contributed to between 20 and 48% of energy which must be considered to be unrealistic *(24–27)*.

8. ETOFIBRATE AND PERIPHERAL ATHEROSCLEROSIS IN NONHUMAN PRIMATES

Fincham et al. evaluated the effect of etofibrate (1.2 ethandiol ester of clofibric and nicotinic acids) on atherosclerosis in Vervets fed an atherogenic diet, with and without etofibrate *(28)*. Total dietary exposure was 38 mo, with etofibrate treatment during the final 27 mo. The etofibrate dose achieved plasma concentrations of clofibric acid comparable to the one achieved clinically.

Etofibrate treatment was associated with consistent significant reductions of LDL-C (–31%) and total cholesterol (–17%) in plasma compared with Vervets who did not receive etofibrate. Necropsy demonstrated lesions equivalent to human atherosclerosis types I–VII, which were compared between treatments both macroscopically and microscopically. Peripheral atherosclerosis was significantly less frequent after etofibrate treatment than in the nonetofibrate group. In aortas a trend for less atherosclerosis after etofibrate treatment was consistent in that it was present for 10 of 12 variables. This result was most obvious for proliferation of smooth muscle and foam cells and accumulation of cholesterol crystals.

9. INFLUENCE OF DIET ON THE METABOLISM OF EICOSAPENTAENOIC ACID (EPA, C20:5, N-3) AND GAMMALINOLENIC ACID (GLA C18:3, N-6)

The effect of diet on the metabolism of EPA and GLA were studied by van Rooyen et al. *(29)* in two groups of Vervet monkeys fed either a WAD (fat 43.5% energy, P:S 0.3, $n = 10$) or a high carbohydrate diet (HCD, fat 20.5%, P:S 3.4%, $n = 10$). Vervets within each dietary treatment were supplemented with either an EPA concentrate or a GLA concentrate for 24 wk, increasing the dose every 6 wk to a maximum of 2400 mg/Kg/d.

The disappearance of EPA and GLA from plasma and erythrocyte membrane (EMB) phospholipids was measured for 12 wk after supplementation was stopped. The authors reported a slower disappearance rate of EPA and GLA from EMB and plasma cholesterol esters (CE) in Vervets consuming the WAD than those consuming the HCD, suggesting a slower metabolic rate of EPA and GLA in Vervets consuming the WD.

Estimations of the rate of disappearance of EPA from the EMB and plasma fractions (t 1/2) after cessation of supplementation showed a t 1/2 of EPA in the EMB-PC of 34 d for the WAD and 22 d for the HCD whereas the corresponding values for EPA in plasma CE was 23 d for the WAD and 14 d for the HCD. In plasma TAG the t 1/2 was 17 d (WAD) compared with 9 d (HCD).

The faster disappearance of EPA from EMB-PC and plasma CE in Vervets on the HCD compared with the WAD is also reflected in the disappearance of EPA from the EMB phosphatidyl ethanolamine (PEA) and EPA and plasma TAG. The t 1/2 of EPA from EMB-PEA was 43 d for the WAD and 31 d for the HCD. It is therefore evident that the disappearance of EPA from EMB-PEA was slower than that measured for EMB-PC. This difference probably results from he fact that the PC fraction is situated on the outside and the PEA fraction on the inside of the EMB *(30)*. Although accurate estimations of the GLA t 1/2 was difficult to access because of the low tissue levels, the disappearance rates of GLA from EMB and plasma also suggested a faster rate of metabolism of GLA in animals consuming a HCD compared with a WAD.

Phospholipid renewal on the outside of the EMB can occur via direct exchange of the phospholipid between the EMB and the plasma lipoprotein, whereas phospholipids on the inside are renewed via acylation of lysophospholipids and could, therefore, take longer to be renewed or metabolized. Faster diasappearance of EPA from plasma TAG than from CE could result from the fact that cholesterol ester transport protein (CETP) has preference for fatty acids in the plasma TAG fraction compared with the CE fraction *(31,32)*. It could also be that the faster disappearance of EPA from the plasma TAG could simply reflect differences in the metabolic rates of LDL and very-low density lipoprotein (VLDL).

The slower rate of disappearance of EPA from the plasma CE in those animals consuming the WAD than those consuming the HCD, suggest a slower metabolic rate of LDL-CE and VLDL-CE in Vervets consuming the WAD. Weight and co-workers *(18)* also reported a faster ^{125}I-labeled LDL disappearance in Vervets on a HCD or prudent diet than those on a WAD.

Measuring disappearance of essential fatty acids (EFA) from tissues after dietary loading, could have potential value as a model for determining the daily requirement for a specific EFA for a particular nutritional situation. During the supplementation with EPA, plasma CE-EPA content was found to increase 60 to 400 times *(29,30)*. However, during the washout period, when a diet deficient in n-3 fatty acids was consumed levels

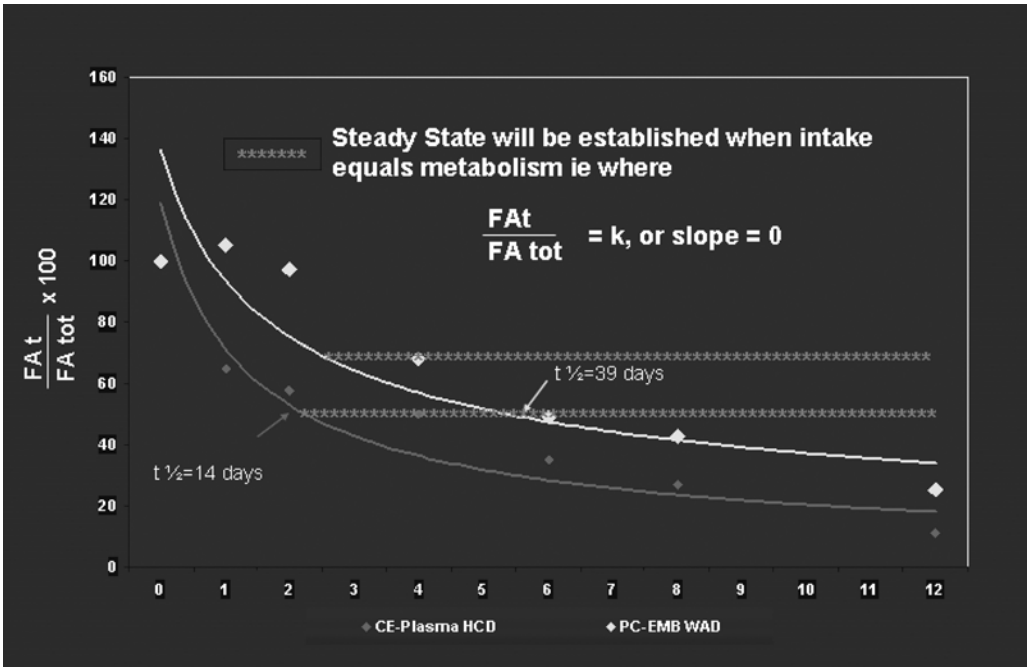

Fig. 1. Disappearance curve of EPA from plasma-CE (HCD) and EMB-PC (WAD).

of EPA declined to reach baseline levels after 12 wk. Tissue levels of EPA under these conditions are thus a function of the intake and metabolism of EPA which is given by the following equation:

$$EPA_T = EPA_I - EPA_M$$

Where T = tissue levels of EPA, I = intake of EPA, M = metabolism of EPA.

Thus, if the intake of EPA exceeds metabolic need, tissue levels will increase whereas when metabolic needs exceed intake, tissue levels will decrease after loading. However, if the supply of EPA tissues metabolic requirements (i.e., $EPA_I = EPA_M$,) tissue levels will remain constant and the entire metabolic process will be in equilibrium. This concept is depicted in Fig. 1.

To determine the EFA requirement for a specific nutritional condition, supplementing an individual or groups of individuals with say EPA to load tissue levels above baseline levels is first required. Second, during the washout phase, supplementation should be continued, but at lower levels in an attempt to establish the amount of EPA required to maintain tissue levels and to find an equilibrium between intake and metabolic needs. That level of supplementation which will maintain steady tissue levels will be an indication of the daily requirement for that specific nutritional condition. This hypothesis however still needs to be evaluated.

10. EFFECT OF GLA AND EPA ON LDL COMPOSITION IN AFRICAN VERVET MONKEYS

Tromp *(33)* studied the effect of GLA and EPA supplementation on the levels and composition of plasma LDL in Vervet monkeys consuming a HCD or a WAD diet

during 24 wk of supplementation. During the supplementation period, the doses of GLA and EPA were progressively increased to reach a maximal dose of between 341 and 422 mg/Kg/d for GLA in the HCD and WAD group and from 318 to 433 mg/KG/d for EPA in the HCD and WAD groups.

Adding GLA to the diet increased the number of circulating particles, but reduced the LDL molecular weight and subsequent depletion of both core and surface molecules in both the HCD and WAD groups.

EPA supplementation to the HCD group decreased the LDL-molecular weight and resulted in minor decreases in LDL-C and LDL-PC levels.

EPA supplementation to the WAD however led to an increase in LDL-C concentration, which mainly resulted from the number of circulating LDL particles. LDL-molecular weight was minimally affected by EPA supplementation.

11. EFFECT OF PALM OLEIN IN A MODERATE-FAT DIET ON PLASMA LIPOPROTEIN PROFILE AND AORTIC ATHEROSCLEROSIS IN NONHUMAN PRIMATES

Dietary fat and cholesterol profoundly affect plasma LDL-C concentrations and saturated fat is implicated as one of the risk factors in hypercholesterolaemia and cardiovascular disease. The cholesterol raising property of saturated fat is generally attributed to palmitic acid, the most abundant fatty acid in diet.

Palm oil is a major source of the world's supply of oils and fats. It has a relatively high content of palmitic acid constituting 43%. Although the cholesterolaemic effect of diets containing palm oil has been the subject of several studies *(34–36)* information on the effect of palm oil on atherosclerosis was lacking.

As shown by Kruger et al. *(20)* LDL-C concentrations in African Vervet monkeys can be modified by the type and amount of fat in the diet. As Vervets are a proven model for both the type and composition for human atherosclerosis lesions *(1,9,23,28)*, van Jaarsveld et al. *(37)* conducted a study to determine the effect of palm oil in a moderate- fat (MFD) 28% E fat; 26 mg cholesterol/1000 KJ) on plasma lipoproteins and the progression of atherosclerosis in a non-human primate model after 25.5 mo of dietary exposure.

Vervets were stratified into three comparable groups. One group received a medium fat diet in which 11% E was derived from lard (AF), a second group was substituted with either sunflower oil (SO) or palm olein oil (PO). Plasma lipids were measured at 6-mo intervals and atherosclerosis was assessed in the aorta and in 5 peripheral arteries after 25.5 mo of dietary exposure. Palm oil relative to SO and AF reduced the risk of developing early lesions in peripheral arteries and aortas (relative to AF). The cholesterolaemic effect of MFD-PO was not significantly different from MFD-SO and MFD-AF.

LDL component concentrations and composition were also assessed at 6-mo intervals. MFD-AF, MFD-SO and MFD-PO groups showed no significant time-specific group differences at 6, 12, 18 or 24 mo with regard to LDL component concentrations, composition, as well as the LDL molecular weight.

Van Jaarsveld and Benadé *(38)* fed 20 vervets a high-fat (34% E) high-cholesterol (98 mg cholesterol/100 KJ) diet (HFD) with a P:S ratio of 0.6 for 6 wk. After 6 wk, the LDL particle size was not significantly different between the HFD and MFD groups,

however, the HFD group had significantly fewer triacylglycerol and significantly more unesterified cholesterol molecules/LDL particle compared with the MFD group. The increased LDL-C concentration seen with the HFD could be accounted for by a more than twofold increase in the number of circulating LDL and not as a result of enrichment of particles with cholesterol.

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