CONTEMPORARY ENDOCRINOLOGY™

Insulin Resistance

Childhood Precursors and Adult Disease

Edited by

Philip S. Zeitler, MD, PhD

Kristen J. Nadeau, MD

Humana Press

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Preface

In the mid 1990s, Drs. Gerald Reaven identified a constellation of clinical findings, known variously as the metabolic syndrome, syndrome X, insulin resistance syndrome or insulin resistance-related disorders, that are associated with an increased risk of heart disease and diabetes. Interest in this topic grew rapidly, culminating in the publication by this series of the book, *Insulin Resistance and the Metabolic Syndrome X*, edited by Drs. Reaven and Laws in 1999. Since the original publication of that now classic volume, the world's population has continued to become more obese and sedentary and the prevalence of disorders related to insulin resistance has continued to increase throughout the developed and developing world.

Of great concern in the last decade is the extension of these deleterious lifestyle patterns to the pediatric population, leading to both obesity and the appearance of insulin resistance-related disorders in youth as well as adults. Today, about one in three children and adolescents in the United States is overweight or obese, and this prevalence approaches one in two among adolescents in certain minority groups. In addition, components of this cardiovascular risk constellation are now being recognized in young adults, adolescents, and even children. Youth are increasingly developing type 2 diabetes, fatty liver disease, hypertriglyceridemia, hypertension, polycystic ovarian syndrome, sleep apnea, orthopedic and psychiatric complications, as well as other complications of obesity and insulin resistance.

Initial reactions to identification of the metabolic syndrome in children have been mixed. Some experts have advocated for early treatment to prevent heart disease, based on pediatric studies showing that atherosclerosis increases as the number of cardiovascular risk factors increase. In contrast, others have argued that the short-term risk of heart disease is low and that treatment, other than lifestyle modification and glucose control should be reserved for adults. However, evidence is now mounting that metabolic syndrome and type 2 diabetes presenting in youth do, in fact, lead to the early appearance of complications. For example, young adults with type 2 diabetes diagnosed during the teenage years are now showing aggressive rates of microvascular and macrovascular disease.

Due to the increasing rates of obesity and insulin resistance, and the associated increased risk of heart disease, the prevalence of CHD is predicted to increase by 5 to 16% in the United States by 2035, with more than 100,000 excess cases attributable to increased obesity among today's adolescents. As a result, experts have predicted

that pediatric obesity may reduce life expectancy in the United States by 2 to 5 years by 2050, which is equivalent to the effect of all cancers combined, making this the first generation to have a shorter lifespan than their parents.

In response to the changing face of insulin resistance and in recognition of advancements in our understanding of the contribution of prenatal, childhood, and pubertal factors, we have asked a number of experts to contribute to a new volume that reflects this updated view. The resulting book presents topics related to insulin resistance and its consequences across the lifespan. In the first section of the book examining epidemiology, our contributors review controversies over the definition of metabolic syndrome in adults and children, current knowledge regarding the epidemiology of insulin resistance in the pediatric population and the contributions of the prenatal and early childhood environment to the development of insulin resistance. The Second part of the book explores pathophysiology, with reviews of the techniques used to study insulin resistance, as well as current knowledge of the molecular and physiologic mechanisms of insulin resistance, including the contributions of adipose tissue and biochemical mediators. This section concludes with discussion of the relationship between insulin resistance and cardiovascular, endothelial, liver and gonadal disorders. The final section of the book explores the impact of exercise and weight loss medications on insulin resistance.

Insulin resistance is likely to be the most important public health concern in many parts of the world in coming decades. Successfully addressing this challenge is going to require an understanding of the molecular, biochemical, and physiological aspects of the disorder, as well as the human face reflected in the epidemiology. This volume provides up-to-date reviews of these areas, providing the reader with a current perspective on issues in insulin resistance as they affect patients across the lifespan. We hope that bringing together these contributions will spur continued interest in the topic on the part of clinicians and researchers, perhaps promoting new points of view and creative approaches to a daunting challenge.

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Part I
Epidemiology

Chapter 1
The Metabolic Syndrome: Definitions, Controversies and Clinical Utility

Lewis W. Johnson and Ruth S. Weinstock

Keywords definitions, diabetes, cardiovascular disease risk

Introduction

Conceptually, metabolic syndrome (MetS) is an insulin-resistant state associated with increased cardiovascular disease (CVD) risk. It has been defined by grouping risk factors, including various combinations of central obesity, glucose intolerance, diabetes, atherogenic dyslipidemia, and hypertension, and is associated with a high risk of developing type 2 diabetes mellitus (T2DM) in individuals without diabetes at presentation. The worldwide obesity and diabetes epidemics and the finding of a high prevalence of MetS in adults and adolescents (1, 2) have greatly increased interest in the definition and potential treatment of this syndrome.

Reports of clustering of CVD risk factors date back to the early 1920's when Kylin described the "hypertension-hyperglycemia-hyperuricemia syndrome"(3). Himsworth, in 1936, differentiated between insulin-sensitive and insulin-insensitive diabetes types, providing the foundation for our current understanding of insulin resistance (4). In 1956, Vague reported the association of upper body (android or male type) obesity with adult onset diabetes, atherosclerosis and gout (5). Eventually, the clinical importance of the association of insulin resistance and CVD risk was recognized by Reaven, who coined the term Syndrome X (6). The clustering of metabolic features associated with CVD risk has also been called the Insulin Resistance Syndrome (IRS) (7) and the Deadly Quartet (8). In 1998 the American Diabetes Association (ADA) described the insulin resistance syndrome as "a constellation of associated clinical and laboratory findings consisting of glucose intolerance, central obesity, dyslipidemia (increased triglycerides (TG)), decreased high density lipoprotein-cholesterol (HDL), and increased small dense low density lipoprotein-cholesterol (LDL), hypertension, increased prothrombotic and antifibrinolytic factors, and a predilection for atherosclerotic vascular disease". They also noted that other insulin-resistant conditions, such as polycystic ovarian syndrome, pregnancy, and glucocorticoid use need to be recognized (9).

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The National Cholesterol Education Program Adult Treatment Panel III (NCEP) in 2001 subsequently recognized MetS as a distinct clinical entity (10). More recently the ADA and the American Heart Association (AHA) have used the term "cardiometabolic risk" instead of MetS (11, 12).

The many definitions of MetS have caused confusion and led to controversy. This chapter will review and compare the five published definitions, the controversy regarding the existence of MetS as a distinct clinical entity, and discuss the usefulness of MetS in estimating CVD risk in clinical practice.

Definitions of The Metabolic Syndrome

In the past decade, five organizations have recommended clinical criteria for the diagnosis of MetS: the World Health Organization (WHO); European Group for the Study of Insulin Resistance (EGIR); National Cholesterol Education Program Adult Treatment Panel III (NCEP); American Association of Clinical Endocrinologists/ American College of Endocrinology (ACE); and, most recently, the International Diabetes Federation (IDF).

The impetus for developing criteria to diagnose this syndrome has been the need to identify persons at increased risk of developing CVD and/or T2DM. The diagnostic criteria used by the various scientific organizations have in general reflected the primary interests of the sponsoring group. The specific criteria for the definitions are summarized in Table 1.1. (13) We will discuss each definition, emphasizing similarities, differences, and clinical usefulness.

World Health Organization (WHO)

The first organization to formalize diagnostic criteria for MetS was the WHO (14, 15). Evidence of insulin resistance is *required* and is identified by the presence of T2DM, impaired fasting glucose (IFG), or impaired glucose tolerance (IGT). For persons with a normal fasting glucose, a euglycemic clamp study with glucose uptake in the lowest quartile for the population is required. In addition to the required evidence of insulin resistance, at least two of the following four risk factors must be present: 1) Obesity, indicated by an increased waist-hip-ratio and/or increased body mass index (BMI) 2) Elevated TG and/or low HDL; 3) Hypertension; and 4) Microalbuminuria. The major criticisms of the WHO definition have been the need to use the euglycemic clamp technique, which is not practical in clinical practice, and the inclusion of microalbuminuria, which can be considered a complication of the syndrome rather than a risk factor. The WHO acknowledged that changes in this definition would be needed as more information became available. These criteria are now infrequently used.

Table 1.1 Working Definitions of The Metabolic Syndrome by Five Organizations[a]

	WHO (1998)[a]	EGIR (1999)[b]	NCEP (2001)[c]	AACE/ACE (2003)[d]	IDF (2005)[e]
Term used	Metabolic syndrome	Insulin resistance syndrome	Metabolic syndrome	Insulin resistance syndrome	Metabolic syndrome
Required factor	• Glucose intolerance, IGT, diabetes mellitus, and/or insulin resistance	• Insulin resistance or hyperinsulinemia (highest 25%)	• Not specified	• At least one of the specified risk factors[f]	• Central obesity (with ethnicity-specific cut-off values-see Table 2)
Additional factors	Plus two or more of the following six:	Plus two of the following four:	Any three or more of the following five:	Plus two or more of the following abnormalities:	Plus any two of the following four factors:
	• Central obesity: waist- to-hip ratio >0.9 in men; waist-to-hip ratio >0.85 in women, and/or BMI >30 kg/m2	• Central obesity: waist circumference 94 cm in men; 80 cm in women • Dyslipidemia: TG >177 mg/dl, HDL–cholesterol <40 mg/dl or treated for dyslipidemia	Abdominal obesity: waist circumference >102 cm in men; >88 cm in women	• Triglycerides: >150 mg/dl • HDL–cholesterol: <40 mg/dl in men and <50 mg/dl in women	• Raised TG level: ≥ 150 mg/dl or specific treatment for this lipid abnormality
	• Raised plasma triglycerides: TG ≥ 150 mg/dl and/or low HDL–cholesterol:	• Low HDL–cholesterol: <40 mg/dl in men; <50 mg/dl in women; or on drug treatment for reduced HDL-cholesterol	• Hypertriglyceridemia TG ≥ 150 mg/dl or on drug treatment for elevated triglycerides	• Reduced HDL–cholesterol: <40 mg/dl in males and <50 mg/dl in females, or specific treatment for this lipid abnormality	• Raised blood pressure: systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg, or treatment of previously diagnosed hypertension
<35 mg/dl in men; or <39 mg/dl in women	• Raised arterial pressure: systolic BP 160 or diastolic BP 90 mmHg; later modified as ≥ 140/90 mmHg	• Hypertension: Systolic BP ≥ 140 or diastolic BP ≥ 90 mmHg, or treated for hypertension	Raised blood pressure: systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg; or on antihypertensive drug treatment in a patient with a history of hypertension	• Blood pressure: systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg	• Raised fasting plasma glucose: FPG ≥ 100 mg/dl, or previously diagnosed type 2 diabetes

(continued)

Table 1.1. (continued)

WHO (1998)[a]	EGIR (1999)[b]	NCEP (2001)[c]	AACE/ACE (2003)[d]	IDF (2005)[e]
• Microalbuminuria: urinary albumin excretion rate ≥ 20 μg/min or albumin: creatinine ratio≥ 20 mg/g; later modified as 30 mg/g	• Hyperglycemia: FPG≥ 110 mg/dl, but non-diabetic	• Raised fasting glucose: ≥ 110 mg/dl); later modified as 100 mg/dl; or on drug treatment for elevated glucos	• vPlasma glucose: FPG 110 - 125 mg/dl; 120 min post glucose challenge (75 g) 140 - 200 mg/dl	

Abbreviations: WHO, World Health Organization; EGIR, European Group for the Study of Insulin Resistance; NCEP, National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III); AACE/ACE, American Association of Clinical Endocrinologists/American College of Endocrinology; IDF, International Diabetes Federation; BMI, body mass index; BP, blood pressure; FGP, fasting plasma glucose;HDL, high-density lipoprotein; IGT, impaired glucose tolerance; TG, triglycerides.

[a]See Refs. 14 and 15.

[b]See Ref. 16.

[c]See Refs. 10, 22, and 26.

[d]See Ref. 28.

[e]See Ref. 29.

[f]Risk factors include the following

(1) overweight: BMI >25 kg/m^2 or waist circumference >40 in for men, >35 in for women; (2) a sedentary lifestyle; (3) age >40 yr; (4) nonwhite ethnicity; (5) a family history of type 2 diabetes, hypertension, or cardiovascular disease; (6) a history of glucose intolerance or gestational diabetes; (7) acanthosis nigricans; (8) polycystic ovary syndrome; and (9) nonalcoholic fatty liver disease.[Adapted]from Metabolic Syndrome and Related Disorders (13), with permission.

European Group for the Study of Insulin Resistance (EGIR)

The EGIR (16) suggested a modification of the WHO definition and a change in name to the IRS. Insulin resistance was required but was defined as a fasting insulin level in the upper quartile of the population. People with diabetes were excluded because one of the major purposes of the definition was to recognize individuals at increased risk of developing diabetes. In addition to the *required* high plasma insulin level, any two of the following risk factors must be present: 1) Central obesity assessed by increased waist circumference (WC); 2) Elevated TG or low HDL; 3) Hypertension; or 4) Fasting glucose ≥110 mg/dl but without diabetes. Unlike the WHO, euglycemic clamp studies were not required, the cutoff for TG was higher, a single HDL cutoff was used for men and women, and WC was deemed a better indicator of abdominal obesity than BMI or waist-to-hip ratio (17). Receiving medical treatment for dyslipidemia and hypertension were also accepted as satisfying the lipid and blood pressure criteria. A major criticism of the EGIR is the use of fasting plasma insulin as a surrogate for insulin resistance, as this test is still not well-standardized.

National Cholesterol Education Program Adult Treatment III (NCEP)

For almost 20 years the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults has been updating clinical guidelines for cholesterol testing and management. In 1988, Adult Treatment Panel (ATP) I identified LDL as the primary target of lipid lowering therapy (18). In 1993, ATP II placed increased emphasis on coronary heart disease (CHD) risk status as a guide to the intensity of cholesterol lowering therapy. Patients with existing CHD or other atherosclerotic diseases were recognized as being at highest risk. HDL was recommended to be included in initial testing and a high HDL was designated as a negative risk factor (19). MetS was recognized as a clinical entity for the first time in the ATP III (10) cholesterol guidelines in 2001. A new definition was proposed in an effort to make the criteria more user-friendly for medical practitioners. The primary goal was to identify individuals at increased risk for CVD.

In contrast to the other definitions, *no single risk factor was required*. Any three of five factors (increased WC, high TG, low HDL, elevated blood pressure, and high fasting plasma glucose) are sufficient to establish the diagnosis. Waist circumference values are higher than those used by the EGIR, are based on the National Institute of Health guidelines for the diagnosis of overweight and obesity (17), and have been shown to increase health risk within normal weight, overweight, and class I obesity BMI categories (20). In other regions of the world, lesser degrees of abdominal obesity are associated with insulin resistance and T2DM (21). Subsequently a Scientific Statement of the AHA/National Heart, Lung, and Blood

Institute (22) indicated that men with a WC of 94 - 101 cm and women with a WC of 80 - 87 cm may have MetS. The blood pressure criteria (systolic ≥130 mmHg or diastolic ≥85 mmHg) are based on The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) classification of pre-hypertension as 120–139/80–89 mmHg (23) and the Framingham Heart Study (24, 25) indicating that people with this range of blood pressure are at increased risk for cardiovascular events. NCEP does not require an indicator of insulin resistance, but instead uses a fasting plasma glucose ≥110 mg/dl and includes individuals with diabetes. The fasting plasma glucose cutoff was later changed to ≥100 mg/dl (26) to correspond to the newer ADA definition of impaired fasting glucose (27).

NCEP attempted to keep its definition simple and easy to apply in clinical practice. The primary goals of NCEP in establishing the criteria for making the diagnosis of MetS were to identify individuals at increased CVD risk and for clinicians to use this information to encourage lifestyle changes to decrease risk. The exclusion of major CHD risk factors (age, gender, smoking, LDL levels), equal weighting of each risk factor, and dichotomous cutoff-points limit the ability of this definition to predict cardiovascular events. Despite these limitations, the NCEP criteria are currently the most commonly used to define MetS.

American Association of Clinical Endocrinologists/American College of Endocrinology (AACE/ACE)

The 2003 AACE/ACE position statement (28) suggested modification of the NCEP criteria with a return to the name “Insulin Resistance Syndrome” (IRS) to emphasize the pathophysiology of the syndrome. They stressed that IRS is a clinical diagnosis that should be considered if any of the risk factors for insulin resistance are present (Table 1.1), in addition to two of the following parameters: 1) Dyslipidemia with cutoffs the same as NCEP; 2) Elevated blood pressure with cutoffs the same as NCEP; 3) Fasting glucose of 110–125 mg/dl (IFG) or IGT defined as a plasma glucose of 140–200 mg/dl two hours after a 75 gram oral carbohydrate load. Persons with diabetes were excluded because a major objective was to identify persons at increased risk for developing T2DM. Their position statement also emphasized that the syndrome not only places an individual at risk for diabetes and CVD, but also for other disease states associated with insulin resistance, including essential hypertension, polycystic ovary disease, nonalcoholic fatty liver disease, certain forms of cancer, and sleep apnea. Compared with the NCEP criteria, the AACE/ACE recognized the limitations of the use of fasting glucose levels and added the use of a 2 hour post-challenge glucose when needed; used BMI instead of WC as a measure of obesity; and increased the number of individuals considered at risk and the list of associated disorders. The ACCE/ACE statement deliberately does not provide a specific definition of the syndrome and allows the diagnosis to rely on clinical judgment.

International Diabetes Federation (IDF)

In 2005 the IDF (29) published new criteria for the diagnosis of MetS. The objective of this new definition was to provide criteria that are applicable in diverse populations. The IDF definition *requires* evidence of central obesity based on measurement of WC with ethnicity based cutoffs (Table1.2). For Caucasian people of European origin, the WC values are the same as those used by the EGIR. Different values are utilized for other ethnic groups, based on available literature (21, 30–32). The WC cutoffs for Japanese were changed to correspond to the same values used for Asians (33). If central obesity is present, then two of four additional criteria are needed to diagnose MetS. Other than the required ethnic-specific increased WC, the IDF criteria are identical to those used in the NCEP definition. If the fasting plasma glucose is above 100 mg/dl, an oral glucose tolerance test is strongly recommended, although not necessary, to define the syndrome.

Metabolic Syndrome Criteria in Adolescents

Age-specific adolescent (12 to 19 years of age) MetS criteria have been derived from the NCEP and IDF definitions for adults (34). Cutoff-points for WC, blood pressure, HDL-chol and triglycerides were developed with Lambda, Mu and Sigma growth curve modeling using data from >6000 male and female participants in the three National Health and Nutrition Examination Surveys from 1988 to 2002. During this time, the prevalence of MetS in the total sample increased from 4.7% to 7.6% according to the adolescent NCEP criteria and from 5.3% to 9.6% according to the IDF criteria. The proposed age-specific cutoff-points for male and female adolescents are shown in Table 1.3.

Table 1.2 Ethnic-Specific Values for Waist Circumference

Ethnic group	Waist circumference
United States (10)	Men ≥102 cmWomen ≥ 88 cm
European	Men ≥ 94 cmWomen ≥ 80 cm
South Asians	Men ≥ 90 cmWomen ≥ 80 cm
Chinese	Men ≥ 90 cmWomen ≥ 80 cm
Japanese	Men ≥ 90 cmWomen Women ≥ 80 cm
Ethnic South and Central Americans	Use south Asian recommendations until more specific data are available
Sub-Saharan Africans	Use European data until more specific data are available
Eastern Mediterranean and Middle East (Arab) populations	Use European data until more specific data are available

From Alberti KGMM, Zimmet P, Shaw J. IDF Epidemiology Task Force Consensus Group: The metabolic syndrome: a new world wide definition. Lancet 2005; 366: 1059–1062.

Table 1.3 Metabolic Syndrome Cut-Points (Percentiles) for Adolescents*

		Waist Circumference (cm)									
		NCEP		IDF		Blood Pressure (mm Hg)		Fasting HDL-chol (mg/dl)		Fasting Triglycerides (mg/dl)	
Age (yrs)	Fasting Glucose (mg/dl)	Male (92nd)	Female (72nd)	Male (83rd)	Female (50th)	Male (92nd/97th)	Female (93rd/99th)	Male (26th)	Female (43rd)	Male (89th)	Female (89th)
12	100	94.2	79.5	85.1	72.5	121/76	121/80	44	48	127	142
13	100	96.2	81.3	87.0	74.2	123/78	123/82	43	48	131	135
14	100	98.0	82.9	88.9	75.7	125/79	125/83	41	49	135	129
15	100	99.5	84.2	90.5	76.8	126/81	126/84	40	49	138	127
16	100	100.6	85.2	91.8	77.7	128/82	128/84	40	49	141	129
17	100	101.4	86.2	92.7	78.5	128/83	128/85	40	49	143	135
18	100	101.8	87.0	93.4	79.2	129/84	129/85	40	50	146	143
19	100	102.0	87.7	93.8	79.8	130/85	130/85	40	50	149	149
20	100	102.0	88.0	94.0	80.0	130/85	130/85	40	50	150	150

*Adapted from Jolliffe and Janssen (34) with permission off cut-point values represent the midpoint of a 1-year increment (ie the values for age 12 represent the values at 12.5 yrs) NCEP = National Cholesterol Education Program Adult Treatment Panel III; IDF = International Diabetes Federation

Controversy

Steadman's Medical Dictionary (35) defines a syndrome as: "The aggregate of symptoms and signs associated with any morbid process, together constituting the picture of the disease". There are 31 pages devoted to short descriptions of >1100 syndromes and, in the past few years, none has stimulated more medical literature and controversy than MetS.

Questioning of the concept of the metabolic syndrome is not new. The first WHO paper in 1998 (14), noted that the person with central obesity, hypertension, and dyslipidemia, with or without hyperglycemia, presents a major classification, diagnostic, and therapeutic challenge and called for a clear description of the essential components of the syndrome with data to support the relative importance of each component. It also noted that internationally agreed upon criteria for central obesity, insulin resistance, and hyperinsulinemia would be of assistance. The most recent IDF definition attempts to establish criteria that can be used worldwide (29).

The controversy heightened in 2005 when the ADA and the European Association for the Study of Diabetes (EASD) published a joint statement questioning the clinical value of MetS (36). Their concerns were: criteria are ambiguous, the rationale for thresholds are ill defined; the value of including diabetes in the definition is questionable; insulin resistance as the unifying etiology is uncertain; there is no clear basis for including/excluding other CVD risk factors; CVD risk value is variable and dependent on the specific risk factors present; CVD risk associated with the syndrome appears to be no greater than the sum of its parts; treatment of the syndrome is no different than the treatment of its components; and the medical value of diagnosing the syndrome is unclear. The authors of the ADA/EASD joint statement suggest a research agenda to critically analyze how the syndrome is defined and to determine its usefulness in predicting CVD risk over and above that of the individual components.

Reaven, who played a critical role in developing the concept of risk factor clustering, has also questioned the clinical usefulness of the term MetS (37–40). His criticisms of the NCEP definition include: obesity is not the result of insulin resistance but rather is a predisposing factor; WC is highly correlated to BMI but much more difficult to measure accurately in an office setting (41, 42); IGT is much better than IFG to identify insulin resistance; and hypertension alone does not indicate insulin resistance. He stresses that insulin resistance is not a disease, but a description of a physiologic state that greatly increases the chances of an individual developing several metabolic abnormalities and associated clinical syndromes.

Grundy, an author of the NCEP definition of MetS (43–46), explains that the purpose of including MetS in the 2001 NCEP ATP III guidelines was to focus attention on the increasing number of obese persons at risk of developing T2DM and CVD. Most of the individuals identified as having MetS are insulin-resistant with a prothrombotic, proinflammatory state and multiple lipoprotein abnormalities, including elevations of apolipoprotein B. Once identified, efforts to improve health through lifestyle changes, particularly weight reduction and increased physical activity, need to be emphasized. The diagnosis of MetS was not intended to be used as a tool to estimate

short-term risk of developing T2DM or CVD, but rather to alert the physician that there may be a long-term risk. He contends that epidemiologic studies have shown that the presence of MetS carries a substantially higher relative risk of both CVD and T2DM.

The National Heart, Lung, and Blood Institute and the AHA (22) continue to support the use of the term MetS as an entity that reflects increased risk of developing CVD and T2DM and support its use as a secondary target for reducing CVD events after the primary targets of smoking cessation, lowering LDL, and blood pressure. They agree that further research is needed to refine the most appropriate therapies for patients with MetS.

The ACE (47) considers the IRS an important clinical entity that predisposes individuals to the development of atherosclerosis and T2DM and may play a role in conditions such as infertility, malignancy, and non-alcoholic fatty liver disease. They specifically distinguish IRS from T2DM and CVD because a major goal was to identify and treat individuals at risk for T2DM and CVD to prevent their development. ACE believes it has been helpful in recognizing the clustering of factors that increase the risk of an individual having IRS, as it has led physicians to search for related risk factors and associated illnesses. Several authorities have also voiced opinions regarding the clinical usefulness of the concept of MetS (48–50).

In 2006, a joint statement from the ADA and the AHA highlighted the importance of CVD risk factor recognition and treatment. The ADA emphasized adoption of a healthy lifestyle, including reduction of obesity and physical inactivity, to decrease "cardiometabolic risk" (11, 12).

Prediction of Type 2 Diabetes and Cardiovascular Disease

Type 2 Diabetes

Because of the obesity epidemic, preventing diabetes and its complications has become a health care priority. Diabetes itself is an important risk factor for CVD, particularly coronary heart disease (CHD) (51, 52), and has been designated as a coronary heart disease equivalent (10).

The identification of subjects at increased risk has become more important because clinical trials have demonstrated that high-risk individuals with IGT ("pre-diabetes") can reduce their risk of progressing to T2DM by more than half with well-structured intensive life style modification (53, 54). However, diagnosis of "pre-diabetes" in these studies has required an oral glucose tolerance test (OGTT), which is time consuming, inconvenient and costly. It would be of value to be able to identify the group at increased risk of developing diabetes utilizing readily available parameters, such as the criteria contained in MetS.

Numerous studies have examined this premise in various populations. In several reports, MetS has been strongly associated with the development of T2DM (55–64). In the San Antonio Heart Study, in a population of mostly Mexican-Americans, the NCEP definition of MetS and IGT had similar sensitivities (59.1%

and 52.8%) for predicting future T2DM, though IGT had a higher positive predictive value (43% vs. 30.8%). The combination of IGT and the NCEP definition increased the sensitivity to 70.8%, with a positive predictive value of 29.7%. Subjects with both MetS and IGT have almost a 60% chance of developing T2DM within 7 years (56).

Using the NCEP definition, a meta-analysis of prospective studies performed from 1998 to 2004 found that the relative risk (RR) for T2DM was 3.0 (60). The Framingham Offspring Study followed 3323 middle-aged adults for the development of new CVD, CHD, and T2DM over an 8-year period and using the NCEP definition, the age-adjusted RR for T2DM was 6.9 for both men and women (61). In the Insulin Resistance Atherosclerosis Study (62) the WHO, NCEP and IDF definitions all predicted the development of T2DM by 3 to 4 fold, compared to individuals who did not have MetS. Although the NCEP definition requires any three of five components, the number of risk factors present is important in predicting diabetes. Using a modified NCEP definition (BMI substituted for WC), the hazard ratios for 1 to ≥4 components were 2.4, 4.5, 7.3, and 24.4 respectively for development of T2DM over a 5 year period (63). Using the WHO definition, Klein (64) found that with one component present, diabetes developed in 1.1% of individuals within 5 years, whereas diabetes developed in 17.9% of those with ≥4 components. Thus, there is little question that MetS identifies a group of individuals with at least a 3-fold increased risk of developing T2DM and the more the components present, the greater the risk.

Not surprisingly, increased blood glucose or hemoglobin A1C levels were most predictive. The FPG level, even in the normoglycemic range, is an independent predictor for developing T2DM among young men and this risk is increased with a higher BMI or elevated triglyceride levels (65). However, the oral glucose tolerance test is inferior to other methods, such as the Diabetes Predicting Model from the San Antonio Heart Study (SAHS), which contains additional important risk factors for T2DM, such as age, ethnicity and family history of diabetes. Adding the 2-hour glucose value to the model, increased the predictive discrimination slightly but involved greater cost and inconvenience (66). The SAHS model has been validated and was found significantly better than the fasting plasma glucose (FPG), but not the 2-hour glucose for predicting 5 year incidence of T2DM in Japanese Americans ≤55 years. The model was significantly worse than the 2-hour glucose and was no better than the FPG in older subjects (67).

The Finnish Diabetes Risk Score (FINDRS) predicts the 10 year risk of developing T2DM with 85% accuracy (68, 69). This T2DM risk assessment model can be obtained at www.diabetes.fi/english/risktest.

Cardiovascular Disease

Although each component of MetS is a risk factor for CVD, there may be other abnormal inflammatory and thrombotic factors that increase risk above the individual components (70). In addition, there are important risk factors not considered in

the definition of MetS, such as LDL level, smoking status, family history and age. It is well-established that the higher the LDL level, the greater the risk for CVD and that lipid-lowering therapy significantly decreases this risk. Focus on MetS should not divert efforts to aggressively address these additional important risk factors.

How well does MetS predict CVD? A meta-analysis of 12 prospective studies using the NCEP definition (some modified by substituting BMI for WC) found a RR of 1.65 for CVD, after adjustment for other cardiovascular risk factors (60). In another meta-analysis that included 37 studies and >170,000 individuals, MetS had a RR of cardiovascular events and death of 1.78. The association was stronger in women and remained after adjusting for traditional cardiovascular risk factors. Substitution of BMI for WC or waist-to-hip ratio did not appear to affect the results (71).

Because approximately one-third of the individuals with MetS have T2DM, it is important to know if persons with diabetes have been included in the analyses of CVD risk in MetS, as the presence of diabetes is considered a CHD equivalent. In an analysis of patients from NHANES II followed for 13 years compared with those without MetS, diabetes or CVD, the hazard ratios for CHD mortality were: MetS (no diabetes) 1.7; MetS with diabetes 2.9; pre-existing CVD without diabetes 3.9; and diabetes with CVD 6.5. Those with only one or two MetS risk criteria were at increased risk for mortality from CHD and CVD (72). In the Framingham study (61), in men, MetS age-adjusted RR were 2.9 for CVD and 2.5 for CHD. For women the RR was lower: 2.3 for CVD and 1.5 for CHD. The risk associated with 2 traits was not substantially increased by having 3 traits and is consistent with the hypothesis that even a modest degree of risk factor clustering reflects an underlying insulin-resistant pathophysiology.

The first prospective population-based cohort study of people with MetS without associated CVD or diabetes, reported a 3 to 4-fold increased risk of CHD mortality, which is as high as in individuals with T2DM (73). However this Finnish study was based on relatively small numbers of events and subsequent results have been less impressive, indicating about a 2-fold increase in CVD mortality (74, 75). This places MetS in the category of a major risk factor but not a CHD risk equivalent. Additional risk stratification is usually needed to direct pharmacological therapy.

Because it may take many years to develop CVD, it is important to review longitudinal studies. A prospective, population based cohort study from Sweden followed men for a maximum of 33 years. When adding MetS to models with established risk factors (smoking, diabetes, hypertension, and serum cholesterol) at age 50, the presence of MetS, as defined by NCEP, significantly predicted total and cardiovascular mortality (HR 1.3 and 1.6). A lag time of 10–15 years occurred before the mortality curves for men with and without MetS separated, indicating that the syndrome predicts a lifetime risk of CVD (76).

In the Multiple Risk Factor Intervention Trial, survivors were followed for an additional median 18 years (77). Those with diabetes or a history of myocardial infarction were excluded. Comparing men with MetS to those without criteria for the syndrome, adjusted HR were 1.2, 1.5, and 1.51 for total, CVD,

and CHD mortality. Among those with MetS, risk was further increased by having more criteria, up to total, CVD, and CHD mortality almost 3 times as high among those with all 5 criteria. Among those with MetS, elevated glucose and low HDL levels were most predictive of CVD mortality, followed by elevated BMI, elevated blood pressure and elevated triglycerides. The results of this study emphasize that the more criteria present, the greater the risk, and that risk increases further with the addition of standard risk factors such as smoking and elevated LDL.

Cardiovascular Disease Risk Stratification

The presumption underlying clinical practice guidelines is that the intensity of treatment (such as the use of cholesterol-lowering drugs and aspirin) should reflect the patient's global risk of developing CHD and CVD (10, 23, 78–83). Many guidelines recommend using the Framingham Risk Score (FRS), which stratifies patients into 3 categories based on an estimated 10-year risk of developing "hard" coronary events (myocardial infarction or death due to CHD). Risk categories are: low risk (<10%), intermediate risk (10–20%), and high risk (>20%). It has been suggested that the intermediate-risk group be reclassified as those at 6% to 20% risk (84, 85). FRS can be calculated by hand using point scores for ranges of age, total cholesterol, HDL, systolic blood pressure and smoking status, using separate tables for men and women or a computerized version can be downloaded to a desktop or handheld computer (www.nhlbi.nih.gov). Although the downloaded version uses continuous variables and the manual version uses categorical variables, the results are nearly identical. Use of either total or LDL cholesterol gives similar results (86). The FRS has been evaluated in several populations and is a good tool for short-term risk assessment and functions well among white and black men and women, but overestimates risk among Japanese-American and Hispanic men and Native American women (87). The FRS has been shown to be a better predictor of short term risk for CVD than MetS (88–90).

Although T2DM has been designated a CHD equivalent, an individual's risk varies depending upon age, duration of diabetes, and the presence of other risk factors. For patients with T2DM, the FRS and other existing risk equations are not accurate, and the United Kingdom Prospective Diabetes Study risk engine (UKPDS) can be used instead (91–93). In addition to age, gender, ethnicity, total cholesterol, HDL, systolic blood pressure, and smoking status, it also includes duration of diabetes and hemoglobin A1C. One problem with the use of the UKPDS risk engine is that it requires the entry of the duration of diabetes, which is not always available. The UKPDS provides risk estimates and 95% confidence intervals in individuals with T2DM for non-fatal and fatal CHD and stroke. This calculator can also be downloaded to a desktop or handheld computer (www.dtu.ox.ac.uk/index.html). A pen-and-paper 10-year risk estimator for T2DM based on the UKPDS risk engine can be employed when a computer is inaccessible (94).

Persons with both T2DM and CHD do not require further risk assessment because they are already considered at "very high risk" and may benefit from the use of more aggressive targets including an LDL goal of <70 mg/dl (95).

The risk factors included in the NCEP definition of MetS, Framingham/ATP III, and the UKPDS Risk Engine are compared in Table 1. 4. MetS is not a good predictor of short-term risk because it does not contain important CHD risk factors such as age, gender, smoking, family history, and LDL cholesterol. However, it does contain an indicator of obesity, which is a long-term risk factor for both CVD and T2DM. The FRS does not include ethnicity, duration of diabetes, and A1C; therefore UKPDS is more appropriate for persons with diabetes.

The ADA offers the Diabetes Personal Health Decision (PHD) cardiometabolic risk calculator on its website (www.diabetes.org/diabetesphd) for use in estimating the risk of developing diabetes and cardiovascular disease. This calculator is based on the Archimedes model, and incorporates age, race/ethnicity, gender, weight, family history, smoking, physical activity, medications, level of fasting blood glucose (A1C if diabetes present), serum lipid level, and blood pressure level. The model is complex, uses differential equations that preserve the continuous nature of biological variables, and must be run on a large network of computers (96–98). This calculator can be used for individuals with or without diabetes or existing CVD, but requires more information than FRS or UKPDS.

Table 1.4 Factors Used in NCEP Metabolic Syndrome Definition, Framingham Risk SCORE/ATP III, and UKPDS Risk Engine to estimate CVD Risk

Criteria Used	NCEP MetS	FRS/ATP III	UKPDS Risk Engine
Abdominal Obesity	X		
Triglyceride	X		
HDL	X	X	X
Blood Pressure	X	X	X
Fasting Blood Glucose	X		
T2DM	X	CHD Equivalent	All Have T2DM
Duration of T2DM			X
A1C			X
Age		X	X
Gender		X	X
Total Cholesterol or LDL		X	X
Smoking History		X	X
Family History of CHD		X	
Ethnicity			X
Atrial Fibrillation			X

NCEP MetS National Cholesterol Education Program Adult Treatment III Definition of Metabolic Syndrome FRS/ATP III ATP III modified Framingham Risk Score; UKPDS United Kingdom Prospective Diabetes Study Risk Engine; LDL Low density lipoprotein HDL High density lipoprotein; CHD Coronary Heart Disease; T2DM Type 2 Diabetes Mellitus

Practical Approach to Estimation of CVD Risk

Persons with CHD or equivalents (diabetes, peripheral arterial disease, abdominal aortic aneurysm, and symptomatic carotid artery disease) are automatically assigned to the high risk category and treated to appropriate evidence-based clinical guideline targets. For other patients with MetS, NCEP ATP III recommends a two-step approach to risk assessment. First, count the number of major risk factors (age, smoking, hypertension, low HDL, family history of premature CHD) present in the individual. Those with ≤1 major risk factor are considered low risk and may be managed with lifestyle modification, although it may be necessary to treat an individual risk factor such as hypertension with medications. Second, for persons with ≥2 risk factors, a 10-year risk assessment is carried out using the FRS. Individuals with a calculated risk of >20% are treated as CHD risk equivalents.

A remaining controversy is how aggressively risk factors should be treated pharmacologically in the intermediate risk group, which includes approximately 40% of the general population (85). The intermediate group with a short-term risk of 10–20% may benefit most from further risk stratification for prediction of future atherosclerotic disease. The goal of additional non-invasive testing is to place as many individuals as possible in the high and low risk groups. Many testing options are available, including calculation of ankle-brachial blood pressure index, assessment of carotid intimal-medial thickness using ultrasound, magnetic resonance imaging, measurement of brachial artery vasoactivity, stress testing, measurement of coronary artery calcium, and several markers of inflammation such as C-reactive protein (CRP). At the present time the most frequently used are measurement of high sensitivity C-reactive protein, assessment of coronary artery calcium, and exercise testing. The cost-effectiveness of performing these additional tests in specific populations is under investigation.

Summary

The five definitions of MetS differ in their ease of use and their usefulness. The WHO, NCEP and IDF definitions include individuals with diabetes. For individuals with diabetes, the designation of MetS has limited usefulness as they are already considered to be at high risk for CVD and need aggressive treatment of all risk factors. The IDF definition requires a WC measurement, which is more difficult to obtain accurately in an office setting. Although abdominal obesity is not required by NCEP, WC is one of the criteria and individuals with 3 of the 5 criteria are almost all obese. It is generally accepted by NCEP guidelines that for individuals with MetS, the risk of developing T2DM is increased about 5-fold and the risk of CVD is doubled. Therefore, the magnitude of increased cardiovascular risk is similar to a major risk factor such as smoking, not a CVD risk equivalent. The favored definition for MetS in the literature currently is the one proposed by NCEP.

Advantages of the NCEP Definition

The major advantage of NCEP is that it is easy to use in office practice (especially if BMI is substituted for WC), all the data are readily available, and no calculations or computer are required. It identifies people at increased risk of CVD and T2DM. Because risk factors tend to cluster, it also alerts the provider to search for additional medical conditions associated with insulin resistance. With the availability of effective drugs to treat hypertension and hyperlipidemia, physicians have tended to pay less attention to behavior modification. MetS has renewed interest in the value of weight reduction and increased physical activity.

Disadvantages of the NCEP Definition

There are several problems with the NCEP definition of MetS: 1) It does not contain important risk factors including age, gender, smoking history, and total or LDL cholesterol, which limits its ability to predict short term risk of CVD. With the exception of age, these risk factors are not as important for T2DM and therefore MetS is better for predicting diabetes than CVD. 2) Dichotomous cutoff-points for blood pressure, blood glucose, TG, and HDL limit the ability to estimate risk. The magnitude of risk is continuous. For example, a systolic pressure of 200 mmHg places an individual at a much higher risk than a systolic pressure of 135 mmHg. 3) Equal importance is given to each of the 5 criteria, although hypertension and low HDL are stronger risk factors for CVD and IGT is more important for risk of developing T2DM. 4) Requirement of 3 of the 5 criteria is arbitrary and imprecise. Risk increases when only 2 factors are present and increases further as more are added. 5) Use of WC to diagnose abdominal obesity may be most accurate but in clinic practice it is seldom recorded. Many researchers have modified the NCEP criteria by substituting BMI for WC. 6) Inclusion of persons with pre-existing CVD or T2DM is probably of little value because they are already considered at high risk.

Conclusion

MetS has provoked much controversy but it has appropriately focused attention on the clustering of risk factors related to obesity and insulin resistance and their association with T2DM and CVD. The major definitions agree on the core components of obesity, insulin resistance, dyslipidemia, and hypertension. The NCEP definition of MetS was never meant to be used as a short-term risk calculator, which is better accomplished using other available tools. The management of low and high risk individuals is well-described but the intermediate risk group may benefit from further assessment using emerging risk markers. Overall, MetS can serve as a simple clinical approach to identify persons for intervention to reduce both CVD and T2DM.

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Chapter 2
Epidemiology of the Metabolic Syndrome and Related Disorders in Children and Adolescents

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Keywords obesity, metabolic syndrome, epidemiology, children, adolescents, waist circumference, insulin resistance

Abbreviations NHANES – National Health and Nutrition Examination Survey; CVD – cardiovascular disease; T2DM – type 2 diabetes mellitus; HDL – high density lipoprotein; LDL – low density lipoprotein; TLC – therapeutic lifestyle change; VLDL – very low density lipoprotein

History of Metabolic Syndrome in Adults

The cluster of hypertension, hyperglycemia, and hyperuricemia was first described in 1923 and studies have since confirmed the clustering of cardio-metabolic factors that has come to be known by many names (1–3). In 1988, Reaven was the first to link type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) to the cluster he called Syndrome X: insulin resistance, hyperglycemia, hypertension, low HDL cholesterol, and raised very low-density lipoproteins VLDL-triglycerides (TG). The concept that cardiovascular risk was increased for individuals prior to the onset of type 2 diabetes gained further support from many studies during the 1990s (4–6). These studies indicated that a "pre-diabetic" state could be identified by the presence of borderline abnormal values of risk factors, such as blood pressure (BP), TG, and HDL cholesterol, that is associated with increased risk for CVD later in life. Dr Paul Zimmet suggested that type 2 diabetes and glucose intolerance were "the tip of the iceberg", arguing that derangements in lipids, BP, and excess weight represent a much larger and unidentified entity lying below the water level (7).

In 2001, the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults published the first major U.S. definition of the metabolic syndrome in the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel (ATP) III). The NCEP ATP III defined the metabolic syndrome as having three or more cardiovascular abnormalities

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that include the following: 1) abdominal obesity or high waist circumference, 2) elevated BP, 3) high TG, 4) low HDL cholesterol, or 5) impaired glucose metabolism. This syndrome is strongly associated with insulin resistance and obesity, but studies also show that there are pro-thrombotic and pro-inflammatory properties associated with this cluster of risk factors (8). ATP III published this definition of the metabolic syndrome to both draw attention to cardiovascular risk factors among overweight and obese adults and improve and enhance the primary care physician's efforts to promote therapeutic lifestyle change (TLC). A number of studies have shown the metabolic syndrome, by the ATP III criteria, to be associated with elevated risk for CVD, T2DM, and overall mortality (9, 10).

The metabolic syndrome has generated considerable controversy about its clinical utility in the adult literature (11–14). However, there is broad agreement that the clustering of cardiac risk factors occurs more frequently than would be predicted by chance alone and that these risks cluster around obesity and insulin resistance. Some argue that the concept of the metabolic syndrome represents a powerful hypothesis that unifies the metabolic components underlying the development of both atherosclerotic CVD and diabetes (12). Others counter that the real question is whether the use of the term provides any useful information beyond what is already known for the individual components or risk factors (14). The literature has also been confusing, because a number of organizations have endorsed different definitions (15, 16) (see Table 2.1 with adult definitions). For the purpose of this paper, the discussion will focus on the NCEP ATP III approach to defining the metabolic syndrome, since most pediatric studies have used variations of this definition.

Table 2.1 Adult definitions for the metabolic syndrome from the 3rd Adult Treatment Panel guidelines, the World Health Organization (WHO), and the International Diabetes Federation (IDF)

WHO, 1998	NCEP, 2001	IDF, 2005
Diabetes, Impaired fasting glucose or impaired glucose tolerance AND any two other factors	Any three of the following five criteria	Central obesity: waist circumference (ethnic specific) AND any two other factors
Elevated blood pressure ≥ 140/≥90 mmHg or Antihypertensive medication	Elevated blood pressure ≥ 130/≥85 mmHg or Antihypertensive medication	Elevated blood pressure ≥ 130/≥85 mmHg or Antihypertensive medication
High triglycerides ≥150 mg/dl	High triglycerides ≥150 mg/dl	High triglycerides ≥150 mg/dl
Low HDL cholesterol <35 mg/dl men, <39 mg/dl women	Low HDL cholesterol <40 mg/dl men, <50 mg/dl women	Low HDL cholesterol <40 mg/dl men, <50 mg/dl women
Obesity, BMI ≥ l30 or	Abdominal obesity, waist circumference	Fasting glucose ≥110 mg/dl or
Waist:hip ratio >0.9 in men	≥ 102 cm (>40 in) men	Previous diagnosed Type 2 diabetes
>0.85 in women	≥ 88 cm (>36 in) women	
Urinary albumin excretion ≥ 20mcg/min or Alb:Cr ratio ≥ 30 mg/g	Fasting Glucose ≥ 100 mg/dl	

Epidemiology of the Cardiovascular Risk Factors in Pediatric Populations:

The clustering of cardiac and metabolic risk factors in children has been described over the last decade (3, 17, 18), and has been closely tied to the epidemic of childhood obesity (19). Reports from the Muscatine Study and the Princeton School Study showed abnormalities in cholesterol and BP associated with obesity that persisted during childhood and adolescence for those who remained in the highest categories of obesity (20–22). Autopsy studies on adolescents and young adults have shown that the extent of fatty streaks and early atherosclerotic lesions is strongly associated with obesity, independent of other cardiovascular risk factors (23–25). Furthermore, the classic study by Berenson showed that the extent of these lesions and streaks increased linearly with the number of cardiac risk factors present (23). These authors found that the risk factors tended to cluster in individual subjects and that the aortas and coronary arteries in those with three or four risk factors had several times greater surface area affected by fatty streaks than those with no risk factors.

The National Health and Examination Surveys (NHANES) have been conducted by the Centers for Disease Control to provide epidemiological and surveillance data for a variety of health conditions. These surveys originally began in the 1960s and collected height and weight data that were used to create the current pediatric growth curves. The survey has been repeated many times over the past four decades and has become increasingly sophisticated, using a complex, multistage sampling design that over-samples the very young, the very old, and ethnic minorities in order to provide a nationally representative sample of the non-institutionalized U.S. population. Use of this complex weighting design allows estimates to be generalized to the U.S. population. In the more recent surveys, cardiovascular risk factors, such as BP, lipoproteins, glucose, and tobacco use, have been collected. The NHANES III survey, collected from 1988 to 1994, was the first to collect waist circumference data for children and adolescents 2–19 years of age, as well as a number of fasting plasma samples, including components of the metabolic syndrome. While several large community cohort studies have collected measures of cholesterol, BP, glucose, and body fat, none provides as representative a sampling of the population as NHANES. The current series of NHANES is a continuous survey that started in 1999 and reports data collected in 2-year cycles. These surveys have been used to describe national rates of obesity (26), trends in abdominal obesity (27), and other cardiovascular risk factors and their distribution across the population (28, 29).

The first report of the metabolic syndrome among teens using an age-adjusted definition from the NCEP found a prevalence of 4.1% in all teens and 28% in overweight teens. Applying the results to census data, this study reported that nearly 900,000 teens during the 1988–1994 period met criteria for the metabolic syndrome (30). When this approach was replicated in the more recent survey of NHANES 1999–2000, the estimate of affected teens had risen to 6%, or nearly 2 million adolescents (31).

While obesity has clearly increased over the last few decades, the changes in other cardiovascular risk factors in children remain unclear. NHANES data indicate that childhood obesity, as defined by body mass index (BMI) ≥95th percentile, has quadrupled from 4% to 17% over the past 20 years (26). National data has also shown that from 1988–1994 to 1999–2000 the *mean* systolic blood pressure (SBP) increased by 2.2 mmHg among those 8–17 years of age. While *mean* TG (−8.8 mg/dl) and *mean* glucose (−2.1 mg/dl) decreased at statistically significant levels, no real change was observed in total cholesterol, HDL, LDL, or glycosylated hemoglobin (28).

Components of the Metabolic Syndrome:

Obesity and Central Adiposity

Obesity among children has skyrocketed to the epidemic proportions that we are facing today. According to the 2003–2004 NHANES data, 17.1% of U.S. children and adolescents are obese (26). The prevalence of obesity among female children and adolescents increased from 13.8% in 1999–2000 to 16.0% in 2003–2004, and the prevalence increased from 14.0% to 18.2% for males over the same time period. The prevalence of obesity differs significantly by race/ethnicity, with the non-Hispanic black population most significantly affected (26).

Body fat in obese children has shown a trend toward a more central distribution, which is usually measured by waist circumference. Ford et al., comparing nationally representative samples of 2–17-year olds, reported that from 1988–1994 to 1999–2002, mean values for waist circumference increased 1.6 cm in male subjects and 2.4 cm in female subjects (28). A report by Li et al. compared rates of abdominal obesity among 2–19-year olds in NHANES III and those in NHANES 1999–2004 (27). They defined abdominal obesity as waist circumference ≥90th percentile for age/sex. They also used a waist circumference-to-height ratio >0.5 as another measure of excess central fat distribution. The prevalence of waist circumference over ≥90th percentile for age/gender had increased from 10% of children in NHANES III to 17% in NHANES 99–04.

Epidemiologic data from England and Spain have shown that changes in abdominal obesity as measured by waist circumference have increased at a greater rate than overall obesity as measured by BMI (32, 33). While central fat was not part of the original syndrome X described by Reaven, its rapid increase and role in accelerating the steps toward premature CVD and T2DM warrant closer measurement in both clinical practice and research.

Adiposity measures for children range from the simple clinical tools, such as weight, height, and BMI percentiles for age/gender, to more precise methods for epidemiology and body composition studies, such as Dual-energy X-ray absorptiometry (DEXA) and bio-impedance analysis (34). BMI percentiles have been the recommended standard for clinical care since the release of the CDC

growth curves in 2000 and is part of the new AMA/CDC expert committee recommendations. Overweight is defined as a BMI from 85th to 95th percentile, while obesity is ≥95th percentile for age/sex. While BMI is a nonspecific measure of mass, the age/sex specific percentile has a high specificity (>98%) for identifying youth with excess body fat (>25–30%) by DEXA scan (35–37). These percentile groups are the recommended cutoff points for identifying children as overweight and obese for clinical use, but no recommendations have been made for measures of central fat. The first papers on metabolic syndrome in youth have applied waist circumference as more specific measures of central fat and have raised the question "should waist circumference be measured in children and adolescents?" Studies show clinical measures of abdominal obesity in children and adolescents to be more specific than BMI for visceral adipose tissue (38–40). Waist circumference has been shown to have added value when examining relationships between adiposity and components of metabolic syndrome or measures of insulin resistance (41). Waist-to-height ratio (WtHR) is another simple anthropometric tool to measure central fat distribution in adults and children (42, 43). European studies show WtHR >0.5 to be associated with adverse cardio-metabolic abnormalities of obesity (44, 45). In a sample of U.S. children 6–19 years of age, 29% of boys and 35% of girls had a WtHR >0.5 in NHANES 99–04, which increased 50% and 65%, respectively, in NHANES III. WtHR, as opposed to BMI percentiles, has been found to be one of the better identifiers of youth with adverse cardiovascular risk factors. Research shows that WtHR could help identify U.S. youth with adverse concentrations of LDL cholesterol, TG, and total cholesterol even among youth who were of normal weight or only overweight (46).

Elevated Blood Pressure

One component of the metabolic syndrome that is an established cardiovascular risk factor is elevated blood BP. Recent guidelines have been revised to increase awareness, screening, and management of hypertension in youth, especially in the face of our current obesity epidemic. BP has increased in children and adolescents over the past decade. NHANES data have shown an increase in SBP of 1.4 mmHg and diastolic BP of 3.3 mmHg from 1988–1994 to 1999–2002. When adjusting for BMI, this increase was reduced by 29% for the SBP and 12% for the diastolic BP. Therefore, although BPs have independently increased, much of the overall increase can be attributed to the increase in obesity among children (28, 47).

Longitudinal data from the Bogalusa Heart Study examined the prevalence of components of the metabolic syndrome in adolescence among adults with prehypertension or hypertension (48). The hypertensive subjects, compared to their normotensive counterparts, showed differences in BP and TG in childhood, and differences in insulin resistance, HDL and LDL cholesterol by adolescence. The authors concluded that obese youth with elevated BP had accelerated, adverse longitudinal changes in risk factors of the metabolic syndrome.

Abnormal Cholesterols: Triglycerides and HDL Cholesterol

The atherogenic dyslipidemia, commonly described with insulin resistance and the metabolic syndrome, is characterized by hypertriglyceridemia, reduced HDL, increased serum VLDL, and increased small-dense LDL (sd LDL) particles. Unlike data on BMI and BP, trends in these lipid components over time are less impressive in youth than in adults and, overall, lipid profiles in children have not changed significantly over the last decade (28). Another report that examined components of the metabolic syndrome in adolescents from the NHANES 99–2000 data applied the cutoffs previously reported by Cook et al.: TG ≥110 mg/dl and HDL ≤40 mg/dl (31). This study found that 23.2% of teens from NHANES 99–2000 had elevated TG, while NHANES III had 23.4%, and low HDL cholesterol affected 23.4% of teens, while NHANES III had 23.3%. The apparent stability of these values may be misleading, because Ford et al. showed that *mean* TG have decreased between these two NHANES series. Therefore, the obesity epidemic may have had an overriding effect on the distribution of lipid abnormalities, despite a decrease in the overall concentration of TG in the whole population.

The sd LDL particle size is also causing concern because prospective studies show that an increased proportion of sd LDL is associated with an increased risk of CVD for a given total and LDL cholesterol. Studies in a population sample of Canadian youth defined the Insulin Resistance Syndrome (IRS) as fasting insulin in the top quartile and two factors: overweight, high SBP, low HDL, high TG, or elevated glucose. They found sd LDL affects 1.2% of normal weight youth (BMI <85th percentile) compared to 6.8% of overweight youth (BMI ≥85th percentile), but only 1% of youth without the IRS had sd LDL, compared to 10% of those with IRS (49). The particularly atherogenic nature of sd LDL particles makes these findings a matter of concern, and the American Heart Association (AHA) now includes discussion of obesity, insulin resistance, and the metabolic syndrome as part of updated cholesterol guidelines for children and adolescents (50).

Impaired Glucose Metabolism

The dramatic rise in obesity over the past two decades has been accompanied by an increase in a number of obesity-related complications, such as impaired glucose tolerance (51) and T2DM (52). In particular, T2DM has increased rapidly over the past 20 years and now represents one-third to one-half of new cases of diabetes among adolescents. The original report of metabolic syndrome among U.S. teens showed that <1% of adolescents had a fasting glucose ≥110 mg/dl. This study excluded children with known diabetes as well as those on medications that could alter glucose metabolism. Since that report, the American Diabetes Association (ADA) has recommended that the cutoff for impaired fasting glucose be lowered to glucose ≥100 mg/dl. In 2000, the prevalence of impaired fasting glucose (≥100 mg/dl) was 1 in 10 boys and 1 in 25 girls among adolescents; the condition affects

1 in every 6 overweight adolescents. This study by Williams and colleagues also used the national sample of teens in the NHANES 99–02 and showed that adolescents who have impaired fasting glucose also have features of insulin resistance and worsened CVD risk factors (53). As of the 99–02 NHANES survey, 0.5% of adolescents were found to have diabetes (of these 29% had type 2 diabetes) and 11% had impaired fasting glucose. This is equivalent to almost 40,000 U.S. adolescents with T2DM and another 2.6 million teens with impaired fasting glucose. These substantial rates are especially important given the high rate of conversion from impaired fasting glucose level to T2DM in adults and the increased risk of CVD in individuals with T2DM (54).

Only a handful of studies have reported the prevalence of impaired fasting glucose with the newer, lower criteria. A community sample of adolescents from Cincinnati reported impaired carbohydrate metabolism, defined as abnormal fasting glucose or impaired glucose tolerance. Overall, 8% had impaired carbohydrate metabolism and over 90% of these subjects had fasting glucose ≥100 mg/dl (55). A smaller study of only obese children from Colorado reported that 12% had fasting glucose ≥100 mg/dl after a 9-hour fast overnight in the hospital (56). Baranowski and colleagues reported the prevalence of abnormal glucose in a cohort of overweight eighth graders at high risk for type 2 diabetes (57). This group was found to have nearly half with a BMI ≥85th percentile and they were mostly from ethnic minority groups (only 15% Caucasian). Impaired fasting glucose (≥100 mg/dl) was found to affect 40% of kids, while impaired glucose tolerance affected 2%. Hence, the increase in affected youth with impaired fasting glucose (glucose ≥100 mg/dl) appears to be a consistent finding that deserves further study of long-term prognostic value for future risk of diabetes, as well as CVD.

Epidemiology of the Metabolic Syndrome in Children

The prevalence of the metabolic syndrome in adolescents (using the ATP III definition modified for age) was first described to be approximately 4%, with nearly 30% of overweight adolescents meeting criteria for metabolic syndrome from 1988–1994 (30). The paper also described demographic differences from a nationally representative sample of U.S. adolescents. Mexican-Americans (5.6%) and whites (4.8%) were the most affected, while African-American teens (2.0%) were the least affected. These racial differences were likely a reflection of the TG and HDL cutoffs applied. Males were more affected than females (6.1% vs. 2.1%). This study also examined the association with family history of heart disease. Of teens with the metabolic syndrome, 7.3% had parents with a positive history of heart attack, compared to 3.6% of teens without metabolic syndrome. These studies suggest that the metabolic syndrome may identify youth at high risk for premature heart disease. However, the cluster may not be as strong an indicator for diabetic risk, since very little difference was found when compared by parent history of diabetes.

A recent report replicated this analysis in NHANES 1999–2000. The authors found an increase in metabolic syndrome over the past decade among all U.S. adolescents (from 4% to 6% in the year 2000) and among obese adolescents (from 28% to 32%). By these estimates, more than 2 million U.S. adolescents have a metabolic syndrome phenotype (31). A significant difference in the paper was the use of a more conservative measure of abdominal obesity, identifying only the top 10% from the current NHANES 1999–2000 sample population. This approach underestimates the prevalence of metabolic syndrome by under-representing the increase in abdominal obesity between these two national samples (27).

The variability of these rates might cast doubts on the validity of any metabolic syndrome definition for pediatric populations (58–60). However, if a pediatric definition is not to be applied, then how many teens overall would meet the adult criteria for the metabolic syndrome? A population study from Cincinnati asked this very question, using both the adult definitions from the NCEP and the World Health Organization (WHO). The prevalence estimate was 4% for all teens using the NCEP and 8% using the WHO definition. Metabolic syndrome among obese teens using these definitions was found to be 20% and 39%, respectively. This study also showed only a fair level of agreement between the two groups identified by these definitions and again raised the question of how to unify the approach in identifying metabolic syndrome in the pediatric population (60).

A literature review of eight pediatric definitions of the metabolic syndrome revealed extensive heterogeneity in the application of the metabolic syndrome definition. A variety of pediatric definitions were applied to a study of 261 black preadolescent females (Girls Health Enrichment Multi-Site studies [GEMS]) and a school-based, cross-sectional study of 240 ethnically diverse preadolescent females (Girls Activity, Movement and Environmental Strategy [GAMES]). Agreement among definitions of metabolic syndrome was poor: the prevalence of MS and cardiovascular risk factor clustering ranged from 0.4% to 23% in the GEMS population and 2% to 25% for the GAMES population with definitions adapted from NCEP ATP III, and 0% to 15% for GEMS and 0.4% to 16% for GAMES using the modified criteria from the WHO (61).

A working group was convened in the summer of 2006 by the National Institute of Child Health and Human Development to examine the metabolic syndrome in children and adolescents (62). A series of findings were reported by leading investigators from across the United States. One study used the most complete national data available from NHANES 1999–2004 to test four major definitions of metabolic syndrome reported in pediatric populations: definitions by Cook (30), Caprio (58), Cruz (59), and the NCEP definition for adults (29). This report showed that the rate of the metabolic syndrome varied from 2% to almost 10% of U.S. adolescents, depending on the definition. Among teens, the definition by Cook yielded a prevalence of 10%, the Adult NCEP definition yielded 4%, while the definitions by Caprio and by Cruz yielded about 2%. When only the obese teens (BMI $\geq$95th percentile) were analyzed, the prevalence rates were 44%, 26%, 14%, and 12%, respectively for these four definitions. The variability of these findings reveals the need for a greater consensus if a pediatric definition is to be considered.

The adult definition has been examined in at least one pediatric longitudinal study. The NHLBI Growth Study reported the prevalence of the metabolic syndrome by the adult NCEP criteria in a prospective group of white and African-American girls. Starting at ages 8–9 and then followed for an average of 10 years, this study showed that less than 1% of girls initially met the adult criteria, but at follow-up, 2.3% of white girls and 3.5% of black girls met these criteria. This study also provided the key insight that early measures of waist circumference and TG level were significant predictors for development of the metabolic syndrome (63).

Two papers by Joliffe and colleagues applied a developmental approach for tracking cardiovascular risk factors in adolescents (64, 65). This group applied the statistical technique known as LMS, used for smoothing population data when multiple cross-sectional studies are combined. This technique was used to develop our current pediatric growth curves by the Centers for Disease Control and Prevention (66). This approach creates age-specific cutoff-points that seamlessly transition with increasing chronologic age until adulthood and allows the investigators to define a specific cutoff-point that can be tracked from the adult value back into younger ages on the curve. For example, the paper on lipoprotein distribution set cutoff-points for borderline high total cholesterol at 200 mg/dl and for high total cholesterol at 240 mg/dl for boys and girls at age 20 (65). National representative data from NHANES III and NHANES 1999–2002 were used to create growth curves for the components of the metabolic syndrome (67). The results for boys and girls are shown in Tables 2.2 and 2. 3, respectively. This paper applied this definition to the NHANES III sample and found a prevalence of 4.7%; this increased to 7.6% when also applied to the NHANES 1999–2002 sample. This paper is limited by the absence of defining criteria for children less than 12 years of age.

This approach was innovative for a number of reasons. It set the HDL values to the gender-specific adult cutoff-points, and it showed how TG differed by gender in the teens 12–15 years of age, likely reflecting some influence of the insulin resistance of pubertal growth. Interestingly, when glucose was set to 100 mg/dl and

Table 2.2 Age-specific values for cardiovascular and metabolic syndrome components that transition into abnormal adult values for boys from U.S. Data.* #

Age (years)	Total Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	WC (cm)	SBP (mmHg)	DBP (mmHg)	Glucose (mg/dl)
12	233	153	127	43.7	94.2	121	76	100
13	225	149	131	42.5	96.2	123	78	100
14	220	145	134	41.3	98.0	125	79	100
15	220	144	138	40.0	99.5	126	81	100
16	223	147	140	39.8	100.6	128	82	100
17	227	151	143	39.8	101.4	128	83	100
18	232	154	146	39.8	101.8	129	84	100
19	238	158	148	39.8	102.0	130	85	100
20	240	160	150	40.0	102.0	130	85	100

* from Jenssen, Circulation, 2006

from Joliffe, JACC, 2007

Table 2.3 Age-specific values for cardiovascular and metabolic syndrome components that transition into abnormal adult values for girls from U.S. Data.* #

Age (years)	Total Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	WC (cm)	SBP (mmHg)	DBP (mmHg)	Glucose (mg/dl)
12	211	136	141	48.3	79.5	121	80	100
13	209	137	135	48.3	81.3	123	82	100
14	208	138	129	48.7	82.9	125	83	100
15	211	139	127	48.7	84.2	126	84	100
16	217	142	129	49.1	85.2	128	84	100
17	225	145	135	491	86.2	128	85	100
18	233	150	142	49.5	87.0	129	85	100
19	238	157	148	49.9	87.7	130	85	100
20	240	160	150	50	88.0	130	85	100

* from Jenssen, Circulation, 2006
# from Joliffe, JACC, 2007

tracked into adolescence, they did not find any variability occurring with age and therefore recommended using the single cutoff-point across all ages and genders. This paper helps address the arguments against the utility of the metabolic syndrome because it provides a developmental approach for tracking cardiovascular risk factors longitudinally from adolescence and into adulthood.

Type 2 Diabetes: End-stage of Metabolic Syndrome or Precursor to CVD?

Some experts have suggested that T2DM is the ultimate expression of the metabolic syndrome, but the two conditions are not synonymous (8, 68, 69). Population studies are currently underway to more closely examine the epidemiology of diabetes during our current obesity epidemic. The SEARCH for Diabetes in Youth study is a prospective surveillance study to ascertain cases of nongestational diabetes in youth <20 years from multiple centers across the United States (70). This study aims to estimate population prevalence and incidence of diabetes according to age, gender, race or ethnicity, and type of diabetes. It also proposes to develop practical approaches to classify diabetes according to the type (type 1 diabetes [autoantibody positive], type 2 diabetes, and mixed or hybrid diabetes).

SEARCH investigators have reported the prevalence of cardiovascular risk factors present in youth with diabetes, and applied the same criteria for components of the metabolic syndrome previously published by Cook et al. (70). Among youth with T2DM, 95% had abdominal obesity (waist circumference ≥ 90th percentile age/sex), 73% had elevated BP, 65% had elevated TG (≥110 mg/dl), and 60% had low HDL (≤40mg/dl). Of subjects with T2DM, 92% had two or more risk factors for the metabolic syndrome, and in multivariate analysis, having T2DM, compared to type 1 diabetes, had a strong, independent association with the odds of having the metabolic syndrome phenotype: odds ratio = 46.8.

These investigators have also described the frequency of lipid abnormalities that exceed target levels for initiating treatment (71). Among youth 10 years or older with T2DM, 33% had total cholesterol ≥200mg/dl, 24% had LDL ≥130 mg/dl, 44% had HDL <40 mg/dl, and 39% had TG ≥150 mg/dl. These categories were taken from the NCEP, AHA, and ADA definitions for optimal lipid concentrations in children and adolescents with both type 1 and type 2 diabetes. Only 5% of these youth were on lipid lowering therapy. This should be of concern since the ATP III guidelines recommend treating T2DM as a cardiac event equivalent. Therefore, youth with T2DM are also felt to be at high risk for premature cardiovascular events such as a myocardial infarction, and should have not only aggressive diet and lifestyle counseling but also pharmacologic management of their lipids (72).

Antecedents of Adult CVD

The awareness of CVD, and its precursors, has gained attention among pediatricians. In addition, a number of studies on other cardiovascular risk factors, such as C-reactive protein (CRP), tobacco, and endothelial dysfunction, have recently appeared in the pediatric literature. CRP has been identified as a key marker of low-grade, chronic inflammation, likely in the vascular bed, that predicts elevated risk of CVD in adults. Ford et al. looked at national data from over 3000 adolescents and found that not only was obesity strongly associated with an elevated CRP, but so were components of the metabolic syndrome (73). Children with abnormal values for cardiovascular risk factors have been found to have increased CRP concentrations as well. Whether differences in CRP concentrations will be useful to assess cardiovascular risk in children and adolescents remains to be established, but the association with obesity makes this a question of interest.

Endothelial dysfunction can be measured by various noninvasive techniques, and offers a physiologic measure of abnormal vascular response. Endothelial dysfunction is an early stage of atherosclerosis, which also predicts CVD. An Italian report examined the association between metabolic syndrome (defined by age-adjusted NCEP criteria) and markers of endothelial dysfunction in Italian youth (74). This study of 100 obese youth showed that those with the metabolic syndrome had greater arterial stiffness than those without it. While no references for these noninvasive measures exist for pediatric populations, these findings suggest that the combination of obesity and other cardiovascular risk factors of the metabolic syndrome has a negative impact on the vascular function in children.

Tobacco still represents one of the greatest preventable causes of mortality in the United States. A report in 2005 analyzed the NHANES III sample of adolescents for the association between passive and active smoke exposure, and the presence of the metabolic syndrome and its components (67). This study used both self-report of smoking and report of a household member who was a smoker to help identify those with active and passive tobacco exposure. In addition, serum cotinine, a metabolite of nicotine, was used to select the cutoff-points for no tobacco exposure

(no detectable cotinine), passive smoke exposure, and active smoking (cotinine ≥15 ng/ml). The study showed that as the levels of tobacco exposure increased, there were increases in the percentage of U.S. teens with low HDL cholesterol, elevated TG, and, perhaps most surprising, abdominal obesity (WC ≥90th percentile). The prevalence of the metabolic syndrome also increased with increasing levels of tobacco exposure. Tobacco and obesity currently represent the leading causes of death in the United States by a wide margin, and now these findings on a population sample of U.S. teens demonstrate that these two major public health threats are converging, with a strong association to the metabolic syndrome, in individuals during only the second decade of life.

Pediatricians once associated CVD in children with congenital heart disease or rarely, genetically linked familial forms of hyper-cholesterolemia. With the recent obesity epidemic and identification of the metabolic syndrome phenotype among children and adolescents this picture has changed. The NHLBI growth study has recently shown that girls who are obese during adolescence are more likely to have increased waist circumference, elevated systolic and diastolic BP, low HDL cholesterol, and elevated TG (75). In multivariate models adjusting for age, the odds that obesity was associated with these factors ranges from O.R. = 3.3 (TG) to 10 (SBP); however, these associations were not found to be significant for total cholesterol (O.R. = 1.2 [0.4 to 3.1]) or LDL (O.R. = 2.4 [0.9 to 6.0]). These results should raise the awareness among pediatricians that obesity is affecting many areas of cardiometabolic health in children and adolescents.

Unfortunately, a number of recent studies from clinic-based samples show that primary care and sub-specialty pediatricians rarely screen for medical comorbidities of obesity. When they do identify these conditions, it is often much later in the course of the disease (76–79). In the office setting, closer surveillance and more accurate assessment of BP, obstructive sleep apnea, hypertension, and impaired glucose tolerance or diabetes appear to be warranted.

Next Step: Clinical Implications

The AHA and the ADA have agreed to move forward on the adult metabolic syndrome debate by remaining jointly committed to a reduction of heart disease and diabetes. While acknowledging many unanswered questions related to metabolic syndrome, they also have recognized the importance of this cluster of cardio-metabolic risk factors in suggesting to providers that they assess all patients for their risk for diabetes and CVD, obesity, elevated BP, dyslipidemia and glucose abnormalities, as well as family history and environmental factors such as smoking, physical inactivity, and poor diet. For all populations, they recommend that lifestyle modification with attention to weight loss and physical activity should be implemented before overt disease develops.

> We strongly recommend that all providers assess patients for their global risk for CVD and diabetes. Despite many unresolved scientific issues, a number of cardiometabolic risk

factors have been clearly shown to be closely related to diabetes and CVD: fasting/postprandial hyperglycemia, overweight/obesity, elevated systolic and diastolic blood pressure, and dyslipidemia. Although pharmacologic therapy is often indicated when overt disease is detected, in the early stages of these conditions, lifestyle modification with attention to weight loss and physical activity may well be sufficient (80, 81).

To treat metabolic syndrome in children and prevent secondary disease development, lifestyle changes around weight management are the first and only choice at this time. While a number of pharmacologic therapies have been tested in adults and teens, the goal of NCEP was to motivate primary care providers to increase their counseling for therapeutic lifestyle changes in overweight or obese subjects in order to reverse the precursors of CVD.

Lifestyle intervention for obesity and the metabolic syndrome components have begun in pediatric populations. A 12-week lifestyle enhancement program was reported to have a positive effect on risk factors for the metabolic syndrome and insulin resistance in overweight youth. Post-interventional evaluations in the group studied showed statistically significant improvements in BMI, SBP, lipids (total, LDL, and TG), postprandial glucose, and leptin levels (82). Weight loss was the targeted outcome, and the reversal of insulin resistance and abnormal components of the metabolic syndrome suggests that intervention early in life is possible and may reduce future CVD risk. The STOPP-T2D Prevention Study is an ongoing multicenter interventional study to develop a school-based, multicomponent intervention trial to prevent T2DM among overweight youth with diabetes, pre-diabetes, or diabetes risk factors (57). This study will test a school-based intervention for impact on obesity and to reverse precursors of diabetes. Pediatric primary care efforts to identify obesity sooner and at a younger age, before abnormalities like the metabolic syndrome develop, are also increasing (83). These parallel efforts show the need to identify more sensitive and diagnostically useful measures of risk beyond overweight or obesity alone. All health professionals who take care of children need to be vigilant for these risk factors, so that intensive work with families to create lifestyle change can be implemented in time to prevent a lifetime of CVD.

Conclusions

The information presented above leads to the practical question – should we define the metabolic syndrome in children? On one hand, obesity is a powerful risk factor for metabolic syndrome. On the other, the lack of an agreed upon definition of metabolic syndrome impedes research and complicates the development of practice guidelines, and may lead to under-diagnosis of cardiovascular risk in certain demographic and clinical populations.

As the pediatric community is just starting to debate the metabolic syndrome definition for children, leading organizations are proceeding with screening and prevention strategies. The National Heart, Lung, and Blood Institute has reissued guidelines for BP screening and management in children (84). The ADA released

a consensus statement for T2DM in pediatrics (85). The AHA has published scientific statements and guidelines for primary prevention of CVD in children (86, 87).

A unifying pediatric definition for clinical purposes is probably premature at this time. However, as research on obesity and future cardiovascular health proceeds, the components identified with the metabolic syndrome need to be examined just as closely as more traditional risk factors, such as total cholesterol and smoking status. (88) Longitudinal studies should be reanalyzed using components of the metabolic syndrome to assess cardiovascular and diabetic risk in greater depth.

While the concept of the metabolic syndrome seems simple for quick clinical identification, it may be a mistake to seek a simple dichotomous definition for such a multifactorial entity. Instead, we need to consider as much data as possible when assessing a child's risk. Bio-statistics and epidemiology have shown us that the use of a more inclusive set of risk factors (biological, genetic, and environmental) and analyses using continuous rather than dichotomous measures, such as those found in a risk score, may help demonstrate whether components of the metabolic syndrome have added value if clustered together or beyond obesity alone. A cumulative risk score that reflects the co-linear nature of all these factors is likely to be more useful than a simple cutoff-point definition. Moreover, whatever criteria are chosen, we must also ensure that the definition is robust enough to endure the transition into adulthood, so that it will be compatible with adult care models and practices (64) (Tables 2.2 and 2.3).

The relationship between risk and future disease is clearly not simple; previous longitudinal studies are unable to account for the current obesity epidemic we are experiencing as a nation. If the "tip of iceberg" today is T2DM, which affects less than 0.5% of teens, there is also a large and growing reservoir of obesity underlying it: the current rate of obesity in adolescents is 17%. Within this group, the presence of a complex set cardiovascular risk factors, hidden below the surface but potentially ready to develop as these adolescents mature, may predict an ominous future for the nation's health.

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Chapter 3
Metabolic Syndrome in Children and Adolescents Worldwide

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Keywords Metabolic syndrome, children, adolescents, epidemiology, prevalence incidence, global

Introduction

Metabolic syndrome (MS) refers to a specific cluster of cardiovascular risk factors, including abdominal obesity, atherogenic dyslipidemia, elevated blood pressure and insulin resistance(1). The importance of the recognition of MS is that it helps to identify those individuals at-risk for both diabetes and cardiovascular disease. The existence of MS in children and adolescents could be especially important since these youngsters may develop the associated secondary cardiovascular complications during the most productive years of their adult life.

Defining the prevalence of MS is important for identifying the public health burden of the condition and for comparing the risks in different populations. Meaningful prevalence estimates, however, require a unified definition. Definitive criteria for adult MS have been proposed by several expert groups and were recently described in detail (2). Briefly, the first definition was established by the World Health Organization (WHO) diabetes group in 1999 (3). The essential components of those criteria were insulin resistance or its surrogates, impaired glucose tolerance (IGT) or diabetes, and at least two of the following: (1) obesity (as measured by a waist/hip ratio >0.9 for males, >0.85 for females or a body mass index [BMI] ≥ 30 kg/m^2), (2) dyslipidemia (triglyceride [TG] levels ≥ 150 mg/dl and/or low high-density lipoprotein cholesterol [HDL-c] <35 mg/dl), (3) blood pressure $>140/90$, and (4) microalbuminuria ≥ 0.30 μg/min.

Another definition was proposed by The European Group for the Study of Insulin Resistance (EGIR) (4). It modified the WHO criteria by excluding people with diabetes, measuring obesity by waist circumference, providing different cutoffs, and excluding microalbuminuria. Thus, hyperinsulinemia is an essential component and two of the following are necessary to be present as well: (1) obesity as measured by waist circumference >94 cm for males and 80 cm for

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females, (2) dyslipidemia TG ≥ 175 mg/dl, HDL-c < 39 mg/dl), (3) blood pressure > 140/90, and (4) hyperglycemia glucose ≥ 110 mg/dl.

The US National Cholesterol Education Program: Adult Treatment Panel III (NCEP/ATP III) criteria (5) facilitated clinical diagnosis by requiring the presence of any three of the following five components: (1) central obesity as measured by waist circumference (males > 102 cm, females > 88 cm), (2) TG ≥ 150 mg/dl, (3) HDL-c < 40 mg/dl for males and < 50 mg/dl for females, (4) blood pressure > 135/85, and (5) fasting hyperglycemia glucose ≥ 110 mg/dl.

The American Association of Clinical Endocrinologists (AACE) proposes yet another set of clinical criteria. These criteria appear to be a hybrid of those of ATP III and WHO. They include BMI ≥ 25 kg/m^2; TG ≥ 150 mg/dL, HDL-c < 40 mg/dL for men and < 50 mg/dL for women, BP ≥ 130/85 mm Hg; fasting glucose between 110 and 126 mg/dL or 2 hours glucose post challenge > 140 mg/dL. Family history of type 2 diabetes, polycystic syndrome, sedentary lifestyle, ethnic group and advanced age are additional risk factors. No defined number of risk factors for diagnosis is specified; diagnosis is based on clinical judgment (6).

The International Diabetes Federation (IDF)(2) recently established a practical definition that allows comparative long-term studies. The essential criterion is central obesity, as assessed by waist circumference, with the same cutoff used by EGIR (i.e., > 94 cm for males and > 80 cm for females). Ethnic-specific waist circumference cutoffs were incorporated into the definition as well. The levels of the other variables were as described by NCEP/ATP III, except for a fasting glucose cutoff of 100 mg/dl.

The problems that arise from the different definitions of the syndrome in adults, from the different cutoff levels of the various parameters of MS, and from the differences between ethnic groups are even more complicated in children and adolescents, for whom no absolute values exist, except for thresholds that change with age. For example, the definition for hypertension changes according to age, gender, and height percentile, and the definition for waist circumference changes with age, gender, and ethnicity. Furthermore, the cutoff values for risk factors, such as hypertriglyceridemia and for low HDL-c, are not well defined in children. Finally, different groups have selected different definitions in modifying the adult criteria. One example is that of the parameter of obesity that is sometimes defined as BMI > 97th percentile, sometimes > 95th percentile, sometimes based on national data, and sometimes according to the Centers for Disease Control (CDC).

The impact of the different definitions of obesity on the estimation of the prevalence of MS was demonstrated in a study among 3036 Iranian children aged 10–19 years (7). Obesity was defined by standardized percentile curves of Iranian children, by the CDC criteria, and by the criteria of the international obesity task force (IOTF). The percentages of subjects determined as having MS among obese children using these alternate criteria were 41.9%, 56.6% and 59.5%, respectively.

Similarly, the significance of different definitions on the estimation of the prevalence of MS was demonstrated in a study among 965 children and adolescents aged 10–18 years from northern Mexico (8). The prevalence of MS according to the EGIR definition was 3.8% compared with 4.5% according to WHO criteria and

6.5% according to NCEP/ATP III criteria. In another cross-sectional study, the implications of variation in MS definitions on MS prevalence estimates were studied in 99 overweight or mildly obese, but otherwise healthy, pre-pubertal 6- to 9-year-olds recruited for a randomized controlled trial of a weight management program (9). Applying the adult NCEP/ATP III criteria, none of the subjects were classified as having MS, while 3% were classified as having MS using modified NCEP criteria. Similarly, the adult EGIR criteria determined that 4% of the children had MS compared to 39% when a modified EGIR for children was used.

The above limitations notwithstanding, this chapter will compare the prevalence of MS in different populations around the world and determine whether the susceptibility to risk factors for MS varies in children from different ethnic groups as has been described in adults. In the preparation of this chapter, the Medline database and Cochrane Library were searched for articles published between September 1978 and August 2006 on MS in children and adolescents, using the following key words in combination: "MS", "syndrome X", "children", "adolescents", "incidence" and "prevalence". Case reports were not included, nor were epidemiological data on MS in children in the USA. Also excluded were data on the prevalence of MS in specific diseases, such as HIV, acute lymphoblastic leukemia, epilepsy, and intrauterine growth retardation.

The identified reports vary considerably: some were from public health organizations with population-based data, while others were series from obesity clinics. Although the results of observed populations in a clinical setting cannot be extrapolated to the general population, we included these reports separately in order to compare the prevalence of MS in different obesity clinics. Some of the reports appeared only in the original languages of the investigators and data were retrieved from the English abstracts. Finally, the definitions used in each study are reported at the beginning of each section.

Epidemiology (Tables 3.1 and 3.2)

Asia

Beijing, China (10)

Definitions. Overweight and obesity were defined by age- and sex-specific BMI classifications for Chinese children and adolescents. Pediatric MS and each risk factor for MS were defined using the criteria for US adolescents: systolic or diastolic blood pressure ≥90th percentile for age and gender, fasting glucose ≥110 mg/dl, TG ≥98 mg/dl and HDL-c ≤50 mg/dl (≤40 mg/dl for boys aged 15–18 years).

The prevalence of MS was studied in the China Nationwide Nutrition and Health Survey (2002 CNNHS), which is a nationally representative cross-sectional survey covering 31 provinces, autonomous regions, and municipalities directly under the

Table 3.1 Prevalence of metabolic syndrome (MS) in children and adolescents in cross-sectional studies worldwide

Country	Study group	Definition of MS	Age (years)	Number (F/M)	Obese (OB) Overweight (OW) Normal weight (NL)	Prevalence 2 out of 5* (%)	Prevalence 3 out of 5* F/M (%)
China (ref 10)	Nationally representative survey	Chinese adjusted NCEP	15–17.9	1160	ALL	19.8	3.3
					OB	100/83	44.4/33.3
					OW	35/45	15/22.6
					NL	18.8/15.5	1.4/1.6
Hong Kong (ref 11)	School children		9–12	271 (120/151)	OB + OW 129	50	8
					NL 142		
Korea (ref 12)	Health survey of a nationally representative sample		10–19	1594 (783/811)	OB 115/139	8.7	3.3
					NL 668/672		
Korea (ref 13)	School children		Elementary school	261 (125/136)	ALL	15.7	2.3
Korea (ref 14)	Observational (families)	Modified NCEP	11–19	229 (103/126)	OW 38/68	24.5	1.9/10.3
					NL 123		
Japan (ref 15)	Screening obese & overweight school children	Japanese adjusted NCEP	6–12	471 (162/309)	OB 299 (102/197)		20.6/16.2.
					OW 172 (60/112)		8.9/8.7
Australia (ref 16)	Tasmanian infant health study		7.4–8.9	298 (208/90)		10.4	5.0
Australia (ref 9)	Overweight children		6–9	99 (64/35)			
Mexico (ref 8)	Cross sectional study	NCEP	10–18	965 (499/466)			6.5

		AACE				7.7
		WHO			All	4.5
		EGIR			OB 268	3.8
					NL 697	26.1
						1.86
Mexico (ref 17)	Cross sectional study		10–15		55	14.5
					110	0
Turkey (ref 24)	Cross sectional study		10–17	1385 (695/690)	OB 68	21%
					NL 1320	2.2%
Poland (ref 28)			14.2 ± 2.5	280	280	50 of children abdominal obesity
				153/127	70 39/31	
Iran (ref 7)	A representative sample of Urban population in Tehran	NCEP	10–19	3036	Overall	
				1623/1413	OB 399	43/41
					OW 382	3/11.4
					NL 2255	3/5

*2 out of 5 risk factors, 3 out of 5 risk factors

Definitions: NCEP, National Cholesterol Education Program; AACE, The American Association of Clinical Endocrinologists; WHO, World Health Organization; EGIR, The European Group for the Study of Insulin Resistance

Table 3.2 Prevalence of metabolic syndrome (MS) among obese subjects in 10 countries

Country	Study group	Definition of MS	Number (F/M)	Age (years)	Obese (OB) Overweight (OW) Normal weight (NL)	Prevalence 2 of 5	Prevalence 3 out of 5 F/M (percents)
Australia (ref 9)	Cross-sectional overweight children		99 (64/35)	6–9			39–60
Brazil (ref 18)	Families with history of Type 2 diabetes mellitus		99	10–19	Overall		6
					OB 30		26
					OW 23		0
					NL 46		0
Chile (ref 19)	Obese children	NCEP	88	8–17	OB 52		34
					OW 19		
					NL 17		
England (ref 20)	Obese children	WHO	103 (67/36)	2.3–18		36	33
France (ref 21)	Obese children	NCEP	308 (166/142)	9–13	Overall		15.9 (17.5/14.1)
		WHO			OB 243		17.3
					NL 65		10.8
Italy (ref 21)	Obese children (obesity clinic) + control group	Modified WHO	1951		OB 588		23.3
					NL 1363		0
Hungary (ref 23)	Obese children (obesity clinic)	WHO	419 (161/258)	12.5 ± 2.4	OB 180	28.3	15
	Control group (outpatient)			13.3 ± 2.2	NL 239	3.8	0.4
Turkey (ref 25)	Obese children (obesity clinic)	WHO	169	7–18			27.2
Greece (ref 26)	Obese children (obesity clinic)		25 (11/14)	6–14	25	11/25	1
					18		
Poland (ref 29)	Obese children		64 (42/22)	12.4 ± 3.4			14

Definitions: NCEP, National Cholesterol Education Program; AACE, The American Association of Clinical Endocrinologists; WHO, World Health Organization; EGIR, The European Group for the Study of Insulin Resistance. OB = obese, OW = overweight, Nl = normal weight

Central Government of China (excluding Hong Kong, Macao, and Taiwan). Data from 1160 children aged 15–17.9 years were available. The overall prevalence of MS was 3.3%. The prevalence of MS in normal weight, overweight, and obese groups was 1.5%, 18.3% and 38.1%, respectively. According to gender, 44.4% of the obese, 15% of the overweight and 1.4% of the normal weight girls had MS compared with 33.3% of the obese, 22.6% of the overweight and 1.6% of the normal weight males. Taken together, 58.3% of the children with MS were overweight or obese. In addition, 83.3% of the obese boys and 100% of the obese girls met two MS criteria, while only 15.5% of normal weight boys and 18.8% of normal weight girls met two MS criteria. Interestingly, four criteria for MS were found in 8.3% of the obese boys, but in none of the obese girls or in the normal weight boys and girls.

Hong Kong (11)

Definitions: Not provided for obesity or any of the tested parameters.

In a cross-sectional study of 271 primary school children between 9 and12 years of age, 129 were obese and overweight. Of the overweight/obese children, 8% had MS: nearly 50% of them met at least two of the three cardiovascular criteria and 77% had insulin resistance, which was best predicted by waist circumference and HDL-c levels.

Seoul, Korea (12)

Three different studies have been conducted in Seoul, Korea.

(1) *Definitions*: The data were defined by modified adult criteria and international BMI cutoffs for overweight and obesity. The cutoff values were: glucose > 110 mg/dl, TG > 140 mg/dl, and HDL-c < 40 mg/dl. Abdominal obesity in children or adolescents was based on waist circumference data that classified subjects with a waist circumference ≥ 90th percentile for age and sex as having abdominal obesity.

In this first study (12), data were obtained on 1594 subjects aged 10–19 years from the Korean National Health and Nutrition Examination Survey 1998, a cross-sectional health survey of a nationally representative sample of non-institutionalized civilian South Koreans. The prevalence of MS was 3.3% in both boys and girls, and 8.7% of the subjects had two components of MS. MS was considered as being strongly associated with elevated alanine aminotransferase (ALT) concentrations in Korean adolescents.

(2) *Definitions:* Abdominal obesity was defined as a value ≥ 90th percentile for age and gender from representative national data of Korean adolescents, a systolic or diastolic blood pressure ≥ 90th percentile for age and gender, fasting glucose ≥ 110 mg/dl, TG ≥ 110 mg/dl and HDL-c ≤ 40 mg/dl.

In this second cross-sectional observational study, the prevalence of MS was examined in each member of 132 Korean nuclear families (13). A total of 229 children aged 11–19 years were studied. The overall prevalence of MS was 6.6%: it was 1.9% among girls and 10.3% among boys. Children with at least one parent with MS were at a 4.1-fold risk for overweight, 3.6-fold risk for abdominal obesity, 5-fold risk for elevated TG, and 4.8-fold risk for MS.

(3) *Definitions:* Cutoff values were as follows: a systolic or diastolic blood pressure ≥ 90th percentile for age and gender, fasting glucose ≥110 mg/dl, TG ≥110 mg/dl and HDL-c ≤40 mg/dl.

In the third cross-sectional study (14), the distribution of the clustering of the metabolic risk was conducted in an elementary school in Seoul, Korea and included 261 children (136 boys and 125 girls). Altogether, 2.3% of the subjects had MS; 7% of these subjects showed clustering of 2 or more metabolic abnormalities, and 0.8% showed clustering of 4 or more. MS was associated with parental obesity and smoking, as well as the child's eating behavior.

Kagoshima, Japan (15)

Definitions: Obesity was defined using international definitions of overweight and obesity. The criteria for MS were adjusted NCEP/ATP III with age-appropriate values obtained from Japanese reference data. The cutoff values were HDL-c <40 mg/dL, fasting serum TG >120 mg/dL, and fasting serum glucose > 100 mg/dL. The 90th percentile values for waist circumference in elementary school children were obtained from Japanese reference data.

The prevalence of MS among 471 obese and overweight elementary school children aged 6–12 years was 8.7% in overweight males, 8.9% in overweight females, 16.2% in obese males and 20.6% in obese females. Normal weight children were not studied.

Australia

Tasmania (16)

Definitions: Fat was measured by the subscapular-to-triceps skinfold thickness ratio. Cutoff values of risk variables were defined as values in the lowest 25% for HDL-c, and highest 25% for insulin, glucose, TG, and systolic and diastolic blood pressure. The co-existence of adverse levels of insulin, glucose, HDL-C, LDL-C, TG, and either systolic blood pressure or diastolic blood pressure was reported.

The clustering of variables of MS was investigated in 298 healthy Australian children (208 boys, 90 girls) in the age range 7.4–8.9 years. A total of 5% of the subjects had a clustering of three risk variables, 2.3% had a clustering of four risk variables and 1% met five criteria.

In this longitudinal study, there was no evidence that infant size predicts development of the insulin resistance or dyslipidemia components of MS by age 8 years. The variables of MS were clustered among the children who had a tendency to accumulate fat on the body trunk.

Adelaide, South Australia (9)

Definitions: Anthropometric and metabolic indicators were classified as normal or elevated using adult- or child-specific cutoff-points. Clustering of MS indicators was also assessed using two adult and three child-specific definitions. The different criteria were chosen according to EGIR adult definition and EGIR modification replacing adult cutoffpoints with child-specific cutoffpoints; and NCEP adult definition. In addition, criteria including fasting insulin with specific cutoffpoint values were also used.

This cross-sectional study looked at the impact of variations in MS definition on MS prevalence estimates in 99 (35 boys and 64 girls) overweight or mildly obese, but otherwise healthy, pre-pubertal 6–9 year olds recruited for a randomized controlled trial of weight management. A total of 0–4% of subjects was classified as having MS when adult definitions were applied. This increased to 39–60% when child-specific definitions were used and varied according to whether hyperinsulinemia was central to the MS classification.

Central and South America

Durango, Mexico (8)

Definitions: In this study, MS was identified using a variety of definitions.

Rodriguez-Moran et al. examined 965 children and adolescents (466 females, 499 males), average age 13 ± 2.6. The prevalence of MS was 6.5% according to NCEP/ATP III, 7.7% according to AACE, 4.5% according to WHO and 3.8% according to EGIR.

In another study from Mexico, the following results were obtained (17).

Definitions: The criteria for MS were based on the NCEP/ATP III criteria. The cutoff values were HDL-c < 40 mg/dL, fasting serum TG > 150 mg/dL, and fasting serum glucose > 100 mg/dL.

The distribution of cardiovascular risk factors among children and adolescents with and without obesity was determined in a cross-sectional study that compared 55 obese and 110 non-obese apparently healthy children and adolescents aged 10–15 years. MS was identified in 14.5% of the obese group and in none of the non-obese children. The cardiovascular risk factors identified in normal weight and obese youngsters were: high blood pressure 4.5% and 6.7%, impaired fasting glucose 6.4% and 14.5%, hypertriglyceridemia 7.3% and 29.1%, and low HDL-c 8.2% and 30.9%,

respectively. The authors concluded that there was a high prevalence of cardiovascular risk factors among even the non obese children and adolescents, and that "metabolically obese" but normal weight individuals can be identified at an early age.

Brazil (18)

Definitions: MS was defined by the presence of at least three of the following: BMI >97th percentile for age and sex, hypertension (blood pressure >95th percentile for age and sex), TG ≥130 mg/dl, HDL-c <35 mg/dl, and insulin resistance HOMA >2.5.

The prevalence of MS was determined among Brazilian adolescents aged 10–19 years with a family history of type 2 diabetes. While the overall prevalence of MS among obese, overweight and normal weight youngsters was 6%, the prevalence in the obese group was 26%, it was not present in the normal and overweight children.

Pontificia, Chile (19)

Definitions: Obesity was defined using international definitions of overweight and obesity. The criteria for MS were adjusted NCEP/ATP III: fasting serum glucose levels >110 mg/dL, hypertension (blood pressure >90th percentile for age and sex, TG >130 mg/dl, HDL-c <45 mg/dl, hyperinsulinemia >20 uU/ml, and insulin resistance HOMA >3.8. Values for waist circumference were based on Chilean norms.

Among 88 Chilean children aged 8–17 years, referred to an obesity clinic, 52 had severe obesity, and 19 were overweight. 17 children with normal weight were the control group. One-third (34%) of the obese children had MS. Hypertension was present in 11.5% of the obese children, elevated TG in 44%, and low HDL-c in 22%. Eleven percent of the severely obese children were glucose intolerant, compared to 0% for the overweight group. Similarly, 67% of the obese children had basal hyperinsulinemia compared with 10% in the overweight group, and 79% had insulin resistance compared with 26% in the overweight group.

Europe

London, UK (20)

Definitions: Obesity was defined as a BMI greater than 95th percentile according to UK 1990 growth references. Hypertension was defined as systolic blood pressure greater than 95th percentile for age and sex. Cutoff values were: HDL-c <35 mg/dl, fasting serum TG levels >155 mg/dl, fasting serum glucose levels >110 mg/dl, and

fasting hyperinsulinemia >15 mU/l for prepubertal children, 30 mU/l for children in mid-puberty and 20 mU/l for children post puberty.

A total of 103 obese children and adolescents 2–18 years of age who underwent assessment of obesity in a joint child and adolescent endocrine service were studied for the prevalence of components of MS according to modified WHO criteria. There were 67 girls (65%). Abnormal glucose homoeostasis was identified in 46% of the study cohort (hyperinsulinism in 40%, impaired fasting glucose in 0.8%, IGT in 11%). Dyslipidemia was identified in 30% and hypertension in 32%. Thirty-one per cent had obesity alone, 36% met two criteria, 28% met three, and 5% met all four criteria. Birth weight, BMI, and family history of MS were not associated with risk of MS. Higher age increased the risk of MS, but MS was identified in 30% of children under 12 years.

Paris, France (21)

Definitions: The definition of MS was derived from the NCEP/ATP criteria and the insulin resistance syndrome (IRS) from the WHO criteria, i.e the presence of IR and two of the following criteria: overweight or obesity, hypertriglyceridaemia, low HDL-cholesterol, impaired glucose tolerance and elevated blood pressure. Hypertriglyceridemia was defined as a value ≥90th percentile for the distribution for age, sex and ethnicity. Low HDL-c levels for all ages and both genders were defined as a value of <40 mg/dl. Elevated systolic or diastolic blood pressure was a value ≥90th percentile for age and sex. Insulin resistance was a HOMA value ≥75th percentile for the distribution for age and sex recently established in a French Canadian population. Glucose tolerance was determined based on the oral glucose tolerance test (OGTT) according to the WHO criteria. Obesity was defined according to the IOTF-recommended international cutoff-points for BMI in childhood for overweight and obesity by sex and age.

A total of 308 overweight and obese children (166 girls, 142 boys) aged 7–17 years, recruited consecutively in the pediatric obesity clinics of three university hospitals, were studied. The frequency of MS was assessed according to the NCEP/ATP III criteria and the frequency of the IRS according to the WHO criteria. The overall frequency of MS and IRS was 15.9% and 42·5%, respectively. The most common component, after abdominal obesity (95·8%) and IR (71·8%), was elevated systolic blood pressure (28.6%). The frequency of glucose tolerance disorders was low (3.6%).

Milan, Italy (22)

Definitions: Modified WHO: glucose intolerance defined as impaired glucose fasting or IGT or diabetes and/or insulin resistance and two or more of the following: (1) Obesity (BMI >97th percentile according to the Italian BMI norms, and waist

circumference ≥97th percentile of control), (2) hypertension (≥95th percentile of control), (3) serum triglycerides (≥95th percentile of controls), and (4) HDL-c (≤5th percentile of control). Microalbuminuria was omitted from the definition.

The prevalence of MS was studied in 588 obese children and adolescents by devising a WHO-derived definition and child-specific criteria; deviation from normalcy was based on an age, sex, and ethnically comparable control group of 1363 subjects. The association of MS with nontraditional cardiovascular disease risk factors was also studied in a subgroup of 206 obese children. The prevalence of MS in obese children was 23.3%. The frequency of MS gradually increased from Tanner stage 1 to Tanner stage 4. Other components of MS, such as uric acid, plasminogen activator inhibitor type 1, and the prevalence of microalbuminuria were higher in obese children with MS compared with obese children without the syndrome.

Pecs, Hungary (23)

Definitions: Obesity was defined as body weight above the expected weight for height by more than 20% and body fat content, calculated from the sum of five skinfold measurements, higher than 25% in males and 30% in females. Hypertension was diagnosed if the average of five blood pressure values was above the 95th percentile for age and sex, and the results of mean arterial blood pressure values of 24-hour ambulatory blood pressure exceeded the 95th percentile value for height and sex. Elevated serum triglyceride was defined as greater than 98 mg/dl (for children <10 years) or 133mg/dl (for children >10 years), while lowHDL-c was defined as ≤35 mg/dl.

The prevalence of MS was studied in 180 (77 females, 103 males) obese and 239 control children. MS was defined as the simultaneous occurrence of hyperinsulinemia, hypertension, IGT and dyslipidemia. Data on the clustering of cardiovascular risk factors in obese and control children were also presented: 15% of the obese children met three criteria (in addition to obesity), compared to 0.4% of the normal weight children. Furthermore, 28.3% obese children met two criteria in addition to obesity compared to 3.8% of the normal weight children. Four abnormalities (termed the metabolic cardiovascular syndrome by the authors) were found in 8.9% of the obese children (8.7% in males and 9.1% in females), but in none of the controls. The duration of obesity was significantly longer among the obese patients with metabolic cardiovascular syndrome.

Ankara, Turkey (24)

Definitions: Modified NCEP: obesity (according to an international reference population based on elevated BMI and not waist circumference), hypertension (defined as blood pressure ≥95th percentile), fasting glucose >100 mg/dL, and HDL-c ≤40 mg/dl.

The prevalence of MS was studied in 1385 apparently healthy students of 10–17 years from Ankara. In this cross-sectional population, 4.9% of the subjects were overweight or obese, and 30 subjects (2.2%) had MS. MS was more common among males (77%). MS was nearly 10 times more common among overweight and obese students (21%), compared with lean students, although 53% of the subjects with MS were neither overweight nor obese. Abnormalities characteristic of MS were highly prevalent even among apparently healthy, lean adolescents.

Konya, Turkey (25)

Definitions: Modified WHO criteria for children: three or more of the following: (1) obesity (BMI >95th percentile based on CDC standards), (2) hypertension (defined as systolic blood pressure ≥95th percentile for age and sex, (3) dyslipidemia (HDL-c <35 mg/dL, fasting serum TG >105 mg/dL for children younger than 10 years or >136 for children older than 10 years, or cholesterol >95th percentile), (4) impaired glucose homeostasis (fasting serum glucose levels ≥110 mg/dl, fasting hyperinsulinemia ≥15 mU/l for prepubertal children, ≥30 for children in midpuberty and ≥20 for post puberty children, or IGT.

This study reported the prevalence of MS and other metabolic abnormalities in 169 obese children and adolescents, aged 7–18 years, referred to pediatric obesity clinics, 100 of whom were prepubertal and 69 of whom were pubertal. MS was identified in 27.2% of them, with a significantly higher rate among adolescents aged 12–18 years (37.6%) than among children aged 7–11 years (20%). There were no significant gender-related differences in the prevalence of MS. There was also no difference in the prevalence of dyslipidemia in prepubertal and pubertal obese patients, though the prevalence of hypertension and hyperinsulinemia was higher in the pubertal group compared to the prepubertal group.

Thessaloniki, Greece (26)

Definitions: Obesity was defined as BMI ≥97th percentile according to Greek growth references charts. Hypertension was defined as systolic blood pressure ≥90th percentile for age and sex. Cutoff values: HDL-c ≤38 mg/dl, fasting serum TG levels ≥130 mg/dl, IGT fasting serum glucose levels ≥126 mg/dl.

The presence of cardiovascular risk factors was studied in a group of 25 obese children and 18 age- and sex-matched control subjects aged 6–14 years. MS was found in one obese subject, and two children met two criteria of MS. Plasma glucose levels during the OGTT were similar in both obese children and control subjects, while plasma insulin levels were significantly higher in obese children.

Odense, Denmark (27)

Definitions: The selected criteria were HDL-c/total cholesterol, plasma triglyceride (>98 mg/dl for children and >115 mg/dl for adolescents), plasma insulin, sum of four skinfolds, and systolic blood pressure. Risk was defined in each gender and age group as the lowest quartile of HDL cholesterol and the top quartile of any of the other risk factors.

Risk factors were determined in a cross-sectional study in 9- to 15-year-old randomly selected boys and girls. MS was not defined since the cutoff-points for the risk factors were chosen arbitrarily, based on quartiles of the population. However, 91 out of 925 (9.8%) participants had three out of five cardiovascular risk factors. Fifty (5.4%) youngsters had four or five risk factors and their individual physical fitness was lower and their BMI higher than the mean values for the population.

Piastow Slaskich we Wroclawiu, Poland (28)

Definitions: Abdominal obesity was defined as waist-to-hip ratio (WHR) ≥0.9/0.85 (male/female), blood pressure ≥130/85, TG ≥150 mg/dl, HDL-c ≤35 mg/dl, hyperinsulinemia ≥20 μUI/ml, and fasting hyperglycemia 110–126 mg/dl.

The presence of MS and family risk factors was studied in 280 (M/F 127/153) obese children aged 14.2 ± 2.5 years with obesity duration of 9.4 ± 4.1years. The control group consisted of 70 (M/F 31/39) normal weight children matched according to age and stage of puberty. MS was reported in 50% of the children with abdominal adiposity. Abdominal adiposity was identified in 66.8%, hypertension in 6.4%, hyperinsulinemia in 30.7%, IGT in 13.2%, fasting hyperglycemia in 5.4%, low HDL-c in 51.4%, and elevated TG in 30.4%.

Katowice, Poland (29)

A study of 64 (42 girls and 22 boys) obese children and adolescents (BMI ≥97th percentile), with mean age of 12.4 ± 3.4 years was conducted. Dyslipidemia was diagnosed in 33 (51.6%) children, hyperinsulinemia or IGT in 10 (15.6%) and hypertension in 12 (18%). Nine (14%) children had MS. The anthropometric predictor for the risk of metabolic complications was a greater waist circumference, while greater hip circumference decreased the risk.

The Middle East

Tehran, Iran (7, 30)

Definitions: MS was defined according to a modified Adult Treatment Panel III definition. Overweight (≥95th percentile) and being at risk for overweight (≥85th–<95th percentile) were based on the standardized percentile curves of BMI suggested for

Iranian adolescents. Abdominal obesity was defined as waist circumference ≥90th percentile for age and sex in this population; elevated blood pressure ≥90 percentile, HDL-c < 40 mg/dl, TG ≥ 110 mg/dl, and elevated fasting plasma glucose (FPG) ≥ 110 mg/dl.

The prevalence of MS was 10.1% in a population-based cross-sectional study of 3036 Iranian adolescents (1413 boys and 1623 girls) 10–19 years of age (boys: 10.3%; girls: 9.9%). Overall, low serum HDL-c and high serum triglycerides were the most common components of MS (42.8% and 37.5%, respectively). Overweight subjects had the highest proportion of MS compared with those at risk for overweight and those with normal weight (boys: 41.1% vs. 11.4% and 3.0%, respectively, $p < 0.01$; girls: 43% vs. 15.2% and 5.0%, respectively, $p < 0.01$).

North America (excluding the USA)

Toronto, Ontario, Canada (31)

Definitions: MS was determined based on extrapolation from ATP-III criteria by the presence of 3 or more of the following 5 criteria: (1) TG ≥ 97 mg/dL; (2) HDL < 46 mg/dl for boys aged 15–19 years, or HDL-c < 50 mg/dl in all other children; (3) fasting blood glucose ≥ 110 mg/dl; (4) waist circumference ≥ 90th percentile for age and sex; and (5) blood pressure ≥ 90th percentile for age, sex, and height. The 90th percentile of waist circumference for age and sex was determined from the NHANES-III.

CV risk factors were evaluated in a population-based study of a Canadian Oji-Cree community involving 236 children aged 10–19 years; 18.6% of the children met the criteria for pediatric MS. As the number of MS component criteria increased, C-reactive protein, leptin, and ratio of apolipoprotein B to apolipoprotein A1 levels rose and adiponectin concentration decreased.

Comment

This review confirms the rise in the global recognition and reporting of MS in children and adolescents (Table 3.1) and demonstrates that the problem of MS is not limited to certain ethnic groups or to particular regions, but has now become essentially worldwide. Since this review is limited to published data, there is no information from regions in which systematic data collection has not yet been carried out and reported. Moreover, the data are difficult to compare across time and across different countries because of the variations between clinical series and public health surveys and because of the inconsistent definitions of MS. Certain characteristics do, however, emerge from the available information.

Prevalence of risk factors in different populations (Table 3.3)

Among US adults, whites of European origin appear to be more predisposed to atherogenic dyslipidemia. US blacks of African origin are more prone to hypertension, type 2 diabetes mellitus, and obesity, while Hispanics, Native Americans and Pacific Islanders appear to be especially susceptible to type 2 diabetes mellitus, but develop hypertension less often than do blacks. By consolidating our accumulated worldwide data, we note that the prevalence of different risk factors appears to vary among children from different origins. Elevated triglycerides were mostly prevalent in children from Iran, Turkey, and Japan, while low levels of HDL-c are reported to be prevalent in children form China, Mexico, and Poland. Hypertension was most prevalent in children from Brazil, England, France and Hungary. However, the data are clearly too limited to characterize and compare European children with Asian or Hispanic children.

In the studies where insulin levels were measured, hyperinsulinemia was reported in >30% of obese children. IGT was relatively rare in most of the studied countries, but was common in Turkey, Poland, and Hungary, and Type 2 diabetes mellitus was common among children in Turkey.

Gender and MS

Again, data are too scarce to arrive at any definite conclusions regarding gender and MS in different parts of the world. A higher prevalence was, however, generally documented in males compared to females among overweight children. For example, the prevalence of MS among overweight children was 5.4-fold higher in Korean males compared to females, 3.8-fold higher in Iranian overweight males compared to females and 1.5-fold higher in Chinese males compared with females. On the other hand, the ratio of MS in overweight Japanese males and females was similar. Interestingly, when considering obese children, a reverse gender ratio is also noted: obese Chinese and Japanese females had a 1.3-fold higher prevalence of MS compared with males, while the ratio was similar for obese Iranian children.

Age and MS

The prevalence of MS and its components was shown to increase with age. In Turkey, the prevalence of metabolic components almost doubled in pubertal children compared to prepubertal children. It is therefore important to specify age groups or Tanner stage groups in order to facilitate comparisons between populations.

Table 3.3 Prevalence of metabolic syndrome components in different populations worldwide

	Country	Central obesity	HT	TG	HDL-c	Hyper insulinemia	Insulin resistance	Hyper-glycemia	IGT	T2DM
Far East	Japan (ref 15)									
	OB	98/97	26.4/24.9	40.2/33.5	8.8/6	60.8/47.7		0/2		
	OW	75/84.8	11.7/10.7	35.8/26.7	8.3/5.4	45/20.5		6.7/0.9		
	Korea (ref 12)	10.6	13	12	10.6			7.9		
	Korean families (ref 13) (F/M)	28.6/43.8	9.1/19.6	21.4/31	4.9/11.9			0/0		
	China (ref 10)									
	OB	88.9/91.7	33.3/46.2	40.6/30.9	59.4/56.4			1.4/0		
	OW	6.8/28.1	25/41.2	28.3/23	66.5/51.8			1.3/1.8		
	NL	1.1/0.3	12.2/16	21.4/14	57.8/54.5			0.6/0.8		
Central & South America	Brazil (ref 18)									
	All		18.2	8.1	8.1		22.2			
	OB		39.5	13	17.4		43.5			
	OW		10	6.7	6.7		23			
	NL		13.0	6.5	4.3		10.9			
	Mexico (ref 8)									
	OB		6.4	29	30.9					
	NL		14.5	7.3	8.2					
	Mexico (ref 17)		7.1	9.5	20.8			7.7		1.8
	Chile (ref 19)									
	OB		11.5	44	22	67	79		11	
	OW		0	20		10	26		0	

(continued)

Table 3.3 (continued)

	Country	Central obesity	HT	TG	HDL-c	Hyper insulinemia	Insulin resistance	Hyper-glycemia	IGT	T2DM
Europe	UK (ref 20) OB		36/25	16/28	10/11	42/36		0/3	11/11	
	France (ref 21)									
	OB + OW	95.8	28.6	22.4	22.1	71.8		1	3.6	
	Poland (ref 28)	66.8	6.4	30	51.4	30.7			13.2	
	Poland (ref 29)		18	51	15.6					
	Hungary (ref 23)									
	OB		41.6/39.8	27.3/44.7	1.3/7.8	53.2/54.4			27.3/28.2	
	NL		3.6/5.2	8.3/6.4	1.2/3.2	4.8/9			0/0	
	Italy (ref 22) OB							0.3	4.1	0.2
	Greece (ref 26) OB		24	24						
	Turkey (ref 24)		15.7	26.7	13.4			0.5		
	Turkey (ref 25)									
	Prepubertal		15	29	14	20		7	19	2
	Pubertal		31.8	44.9	20.2	43.7		14.4	27.5	4.3
Middle East	Iran (ref 7)									
	Overall	10	23.8	37.5	42.8			0.6		
	OB	57.3	42.4	63	56.8			1.3		
	OW	12.5	27.5	50.6	52.8			1		
	NL	1.6	20	30.9	38.7			0.5		

Definitions: OB = obese, OW = overweight, Nl = normal weight

MS in Normal Weight Children

Several authors reported the existence of MS and components of MS in normal weight children. Our literature search revealed that 1.5% of normal weight Chinese children, 1.9% of normal weight Mexican children, 2.2% of normal weight Turkish children and 3% and 5% of normal weight Iranian females and males, respectively, have MS.

Summary

The prevalence of MS ranges between 3–6% of all children in cross-sectional studies. However, MS occurs in 20–45% of obese children and adolescents. The prevalence of different risk factors varies among children from different populations worldwide.

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Chapter 4
Maternal-Fetal Contributors to the Insulin Resistance Syndrome in Youth

Dana Dabelea

Keywords insulin resistance, youth, obesity, intrauterine, sensitive period

Introduction

The life course approach to chronic diseases considers fetal life a critical period for the development of later, adult chronic diseases (1). Numerous studies have linked growth restriction *in utero*, evidenced by the measurement of low birth weight or thinness at birth, with an increased risk for the metabolic syndrome (IR syndrome) (2), insulin resistance (IR) (3), poor glucose tolerance or type 2 diabetes (T2DM) (4), and cardiovascular disease (CVD) (5, 6) later in life. The effects were strong and greatly enhanced by the presence of adult obesity. These findings were linked to poor fetal nutrition during intrauterine life and constitute the basis for "the thrifty phenotype" hypothesis (7). At the other end of the birth weight distribution, macrosomic infants of women with diabetes during pregnancy are a group exposed to over-nutrition during the intrauterine life. They have also been shown to have an increased risk for obesity and T2DM as adults (8, 9).

The concept of the metabolic or IR syndrome as a distinct entity is controversial. However, it is a useful construct that includes a cluster of pathologies: obesity, abnormal glucose tolerance, dyslipidemia, and hypertension (10). Interest in the IR syndrome in the pediatric population has been driven by increasing rates of overweight and obesity, particularly among youth (11). Current estimates suggest that more than 2 million adolescents, most of whom are overweight, have an IR syndrome phenotype (12). This number is expected to rise along with the prevalence of overweight and obesity in this age group, leading to increasing prevalence and earlier onset of morbidity and mortality (13, 14). For many years considered a disease found only in adults, T2DM is now present and increasing much earlier in life, in children and adolescents (15,16), in close temporal association with an epidemic of obesity affecting all ages (17, 18). Given the increased frequency with which adult chronic conditions are now observed among children, exploring whether and how fetal exposures *in utero* may contribute to the complex picture of the IR syndrome in youth is of considerable importance.

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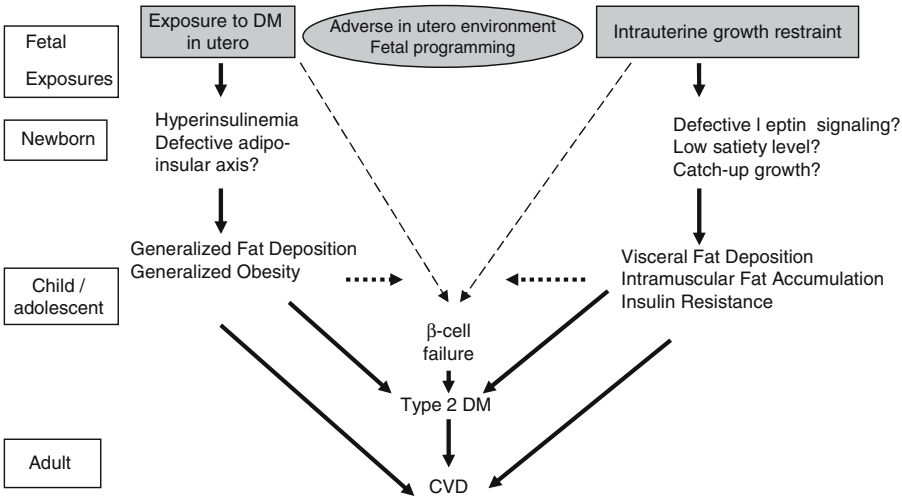

Fig. 4.1 The perinatal road to future chronic diseases: DM = diabetes mellitus; CVD = cardiovascular disease

Figure 4.1 summarizes recent concepts and hypotheses pertinent to the perinatal road to later chronic diseases. This chapter summarizes the available literature on the effects of two main fetal exposures on components of the IR syndrome in youth: (a) exposure to diabetes *in utero* and (b) intrauterine growth restraint. Also included is a discussion of potential mechanisms through which these exposures might operate.

Exposure to Diabetes *in utero*

The hypothesis of fuel-mediated teratogenesis (19) proposes that intrauterine exposure to an excess of fuel (for example, glucose) causes permanent fetal changes. In pregnancies complicated by diabetes, this would lead to malformations, greater birth weight, and an increased risk of developing T2DM in later life. Recently, obesity in the offspring has been included as an outcome in pregnancies complicated by diabetes. The hypothesis is now widely accepted, although the relatively few studies that have examined the question are poorly comparable and focus almost exclusively on growth and glucose regulation.

Effects on growth, adiposity, and risk for childhood obesity

Development in a diabetic intrauterine environment results in excess fetal growth. While maternal glucose freely crosses the placenta, maternal insulin does not (19). The developing fetal pancreas responds to this increased glucose load by producing

additional insulin, which in turn acts as a fetal growth hormone promoting growth and adiposity.

Only two studies have prospectively examined the role of exposure to diabetes *in utero* on childhood growth and later obesity, the Pima Indian Study and Northwestern University Diabetes in Pregnancy Study. The offspring of Pima Indian women with pre-existent T2DM and gestational diabetes (GDM) were larger for gestational age at birth, and at every age they were heavier for height than the offspring of pre-diabetic (women who did not have diabetes before or during the index pregnancy but developed diabetes later) or non-diabetic women (8, 20, 21). Relative weight in these latter two groups was similar. From these data, it is not clear whether the diabetic intrauterine environment leads to childhood obesity directly or simply results in a high birth weight, which in turn leads to childhood obesity. However, in offspring of diabetic pregnancies with normal birth weight, childhood obesity was still more common than among offspring of non-diabetic pregnancies (22). Even the normal birth weight offspring of the Pima Indian diabetic women were heavier by age 5–9 years than the offspring of non-diabetic and pre-diabetic women.

The Diabetes in Pregnancy Center at Northwestern University in Chicago has conducted the only other longitudinal study, which reported excessive growth in non-Hispanic white (NHW) offspring of women with diabetes during pregnancy, mostly GDM and insulin-treated DM (23). Children included in this study were examined at birth, at age six months, and annually to age eight years. The symmetry index (an obesity index), which was normal at one year of age, increased during follow-up so that by age eight years the mean symmetry index was almost 1.3; that is, the children were, on average, 30% heavier than expected for their height.

Data outside the Pima Indian population are scarce, but most suggest that offspring of diabetic pregnancies are tall and heavy (24–26). Most studies have not systematically had a longitudinal follow-up from birth of the offspring of diabetic women, but do provide evidence that these offspring are prone to obesity. In 1959, Hagbard et al. (27) reported the stature of 239 children with an average age of 5 years who were born after the onset of their mothers' diabetes and 68 with an average age of 16 years who were born before the onset of the diabetes. Since the two groups of children were of quite different ages, each was compared with age-appropriate normal data. Those born after the mothers got diabetes were significantly heavier than normal for their age, while those born before showed no deviation from normal. Weiss et al. (25) studied the offspring of women with type 1 diabetes (T1DM) and reported that they also had a significantly higher body mass index (BMI) and symmetry index than the offspring of control women. The measures of obesity were significantly correlated with fasting and post-load blood glucose. This suggests that the long-term effects of the diabetic intrauterine environment on the body size of the offspring are similar regardless of whether the mother has T1DM, T2DM, or GDM.

Recent data indicate that the increase in birth weight experienced by offspring of diabetic mothers may represent an increase in the ratio of fat mass to fat-free mass (28). Using total body electrical conductivity (TOBEC) estimates of body composition, Catalano et al. (28) showed that neonates born to women with GDM

have 20% higher body fat (436 vs. 362 g) compared with neonates born to women with normal glucose tolerance, at similar birth weight. These findings provide evidence of early effects of exposure to diabetes *in utero* on neonatal obesity risk, effects that, as shown above, may be amplified throughout the life course.

There is substantial evidence that the excess growth experienced by offspring of diabetic mothers is not due to genetic factors alone, but is also caused by an abnormal intrauterine environment. First, obesity is no more common in the Pima Indian offspring of women in whom diabetes developed after delivery than in those of non-diabetic women (8, 29). Second, obesity in the Pima Indian offspring of diabetic women cannot be accounted for by maternal obesity (22). Third, the excessive growth seen in the offspring of diabetic mothers is not found in offspring of diabetic fathers (30). Fourth, within Pima Indian families with non-diabetic offspring, BMI was significantly higher (+2.6 kg/m^2) in the 62 siblings exposed to their mothers' T2DM during pregnancy (the diabetic intrauterine environment) than in the 121 siblings born before maternal diabetes was diagnosed (31). In contrast, there was no significant difference between siblings born before or after their father was diagnosed with T2DM (mean BMI difference: 0.4 kg/m^2) (31). These data support the hypothesis that exposure to DM *in utero* has effects on offspring body size that are independent of, or in addition to, genetic susceptibility to obesity. Data on 9–14-year-old non-Hispanic white children in the Growing Up Today Study (26) showed, however, that the association between a history of maternal GDM and adolescent overweight was substantially attenuated after adjustment for reported maternal BMI, suggesting that genetic susceptibility for obesity does account for part of the observed association in this population. The authors concluded that their results were consistent with GDM programming the fetus for later, postnatal influences that lead to obesity, although they did not implicate GDM as a sufficient cause of offspring obesity.

Effects on glucose–insulin metabolism and risk for T2DM

Exposure to diabetes *in utero* has also been shown to have long-lasting effects on glucose–insulin homeostasis. The Diabetes in Pregnancy Center at Northwestern University enrolled offspring of women with pre-existing diabetes (both insulin dependent and non-insulin dependent) and gestational diabetes from 1977 to 1983. Plasma glucose and insulin were measured both fasting and after a glucose load yearly from 1.5 years of age in offspring of diabetic mothers and one time at ages 10–16 years in control subjects (23). At the age of 12 years, offspring of diabetic mothers had significantly higher glucose and insulin concentrations and higher prevalence of impaired glucose tolerance (IGT) than the age-and sex-matched control group (19.3% vs. 2.5%), and two female offspring had developed T2DM. In this cohort, the predisposition to IGT was associated with maternal hyperglycemia, regardless of whether it was caused by GDM or pre-existing insulin-dependent or non-insulin-dependent diabetes (20).

A significant correlation between the 2-h post-load plasma glucose in 15–24-year-old Pima women and their mothers' 2-h glucose during pregnancy has been described (32). By ages 5–9 and 10–14 years, T2DM was almost exclusively present among the offspring of diabetic Pima Indian women (33). In all age groups there was significantly more diabetes in the offspring of diabetic women than in those of pre-diabetic and non-diabetic women, and there were much smaller differences in diabetes prevalence between offspring of pre-diabetic and non-diabetic women. These small differences may be due to differences in the genes inherited from the mothers, while the large difference in prevalence between the offspring of diabetic and pre-diabetic mothers, who have presumably inherited the same genes from their mothers, is the consequence of exposure to the diabetic intrauterine environment. These differences persisted after adjusting for the presence of obesity in the offspring, therefore suggesting that the effects of exposure to diabetes *in utero* on offspring's glucose metabolism are not entirely mediated through the development of obesity in the exposed offspring. Moreover, within the same family, siblings born after the mother's diagnosis of diabetes were 3 times more likely to develop diabetes at an early age than siblings born before the diagnosis of diabetes in the mother. Since siblings born before and after diabetes diagnosis carry a similar risk of inheriting the same susceptibility genes, the different observed outcomes reflect the effect of intrauterine exposure to hyperglycemia (31).

Evidence also exists that among mothers without preexistent DM or frank GDM fetal exposure to mildly elevated maternal blood glucose concentrations that are below the current cutoff-points used to diagnose GDM is still important. A recent study in Pima Indian pregnant women who were not diabetic and had glucose levels in the '"normal' range" found a direct linear association between maternal fasting glucose during the third trimester of pregnancy and risk of T2DM in their offspring, as well as confirming a linear association between maternal glucose and offspring birth weight in non-diabetic pregnancies (34). In contrast, the EarlyBird Study, a historical prospective study in the United Kingdom, reported no relationship between maternal highest glucose level in pregnancy and child's birth weight, or the child's weight and estimated insulin resistance at 8 years (35).

In Pima Indian children aged 5–19 years, the prevalence of T2 DM has increased two- to three-fold over the last 30 years (33). The percentage of children who have been exposed to diabetes *in utero* has also increased significantly over the same period, which was associated with a doubling of the incidence of diabetes in children attributed to this exposure (from 18.1% in 1967–1976 to 35.4% in 1987–1996). The "epidemic" of T2DM in Pima Indian children was almost entirely accounted for statistically by the increase in exposure to diabetes during pregnancy and the increase in obesity. Exposure to intrauterine maternal hyperglycemia was the strongest single risk factor for T2DM in Pima Indian youth (odds ratio 10.4, $p < 0.0001$) (33). The effects of maternal diabetes on the child may therefore be viewed as a cross-generational vicious cycle (36). Children whose mothers had diabetes during pregnancy are at increased risk of becoming obese and developing diabetes at young ages. Many of these female offspring already have diabetes or

abnormal glucose tolerance by the time they reach their childbearing years, thereby perpetuating the cycle.

Whether the vicious cycle of the diabetic pregnancy is operating in populations other than American Indians has not yet been studied. Two recent studies among members of the Kaiser Permanente Health plan show a significant increase in the cumulative incidence of GDM: ~35% over the last decade in Northern California (37), and 11% annually between 1994 and 2002 in Colorado (38). Important and disturbing, both studies show increasing rates of GDM among all racial/ethnic groups. It is, therefore, very likely that the vicious cycle of diabetes in pregnancy initially described among Pima Indians is also operating among other U.S. racial/ethnic groups. Exploring the effects of exposure to diabetes *in utero* on fasting glucose and insulin concentrations among youth of other racial/ethnic groups than American Indians is of considerable importance.

The mechanisms by which exposure to diabetes *in utero* increases the risk of IGT and T2DM are still uncertain. A higher frequency of maternal than of paternal transmission of diabetes has been demonstrated in GK rats (39). In these rats the diabetic syndrome is produced by strepotozotocin injection or glucose infusion. They do not have any genetic predisposition for diabetes, nor can their diabetes be classified as type 1 or 2. These studies have demonstrated that hyperglycemia in the mother during pregnancy leads to impairment of glucose tolerance, and decreased insulin action and secretion in adult offspring (40). Several studies performed in newborns of diabetic mothers have shown an enhanced insulin secretion to a glycemic stimulus in these neonates (41), and consistent with these findings Van Assche (42) and Heding (43) described hyperplasia of the β-cells in newborns of diabetic mothers. Whether this is a transient phenomenon, or leads to impaired glucose tolerance later in life when insulin resistance becomes important, is still uncertain. Impaired insulin secretion has also been proposed as a possible mechanism. Among Pima Indian adults the acute insulin response was 40% lower in individuals whose mothers had DM during pregnancy than in those whose mothers developed DM after the birth of the subject (44). On the basis of observations made in rats and supported by the Pima Indian findings, it may be hypothesized that exposure to hyperglycemia during critical periods of fetal development "programs" the fetus to later develop insulin resistance and defective insulin secretion, although the sequence of metabolic disturbances is less clear. Importantly, these effects are independent of birth weight (22, 45, 46), they appear to be similar regardless of mother's type of diabetes (25, 47), and they may not be entirely explained by the development of obesity in exposed offspring (20, 48).

Effects on the adipoinsular axis

As shown in Fig. 4.1, there is a suggestion that relative hyperinsulinemia in offspring of diabetic pregnancies may be a precursor to childhood obesity. Amniotic fluid insulin concentrations have been shown to correlate positively with childhood

obesity among these offspring (49). In the Diabetes in Pregnancy study, amniotic fluid insulin was collected at 32 to 38 weeks of gestation. Among 6-year-old offspring, there was a significant positive association between the amniotic fluid insulin level and childhood obesity, as estimated by the symmetry index. The insulin concentrations in 6-year-old children who had a symmetry index of less than 1.0 (86.1 pmol/l) or between 1.0 and 1.2 (69.9 pmol/l) were only half of those measured in the more obese children who had a symmetry index greater than 1.2 (140.5 pmol/l, $p < 0.05$ for each comparison).

However, the mechanisms through which the exposure to a disturbed fuel environment during intrauterine life might predispose to later adiposity are largely unknown. Leptin, a hormone secreted by adipocytes and by the placenta, is important for fetal growth (50). In umbilical cord blood, there is a strong increase in leptin levels coinciding with the development of fetal adipose tissue. Insulin may increase leptin levels, and after birth there is a functional negative feedback loop between leptin and insulin (adipo–insular axis). Elevated cord blood leptin concentrations were found in both infants of T1DM (24.7 ng/ml) and GDM mothers (29.3 ng/ml) as compared to controls (7.9 ng/ml) (51). Offspring of mothers with preexistent DM had a higher ponderal index at birth, as well as higher cord blood insulin and leptin levels, than those of mothers with GDM or control subjects (52), reflecting the influence of maternal hyperglycemia on fetal growth. Cord blood leptin appears to reflect, therefore, fetal growth in newborns of diabetic mothers. It appears to be a useful marker of fat mass at birth and it quantifies even a "mild diabetes effect" on the newborn. In two other studies, exposure to GDM *in utero* was associated with both hyperleptinemia and hyperinsulinemia in the newborns (53, 54), suggesting that the exposure may lead to increased insulin secretion and adiposity in the fetus, which may reflect an inability of rising plasma leptin concentrations to control the release of insulin (54). Fetal over-nutrition may therefore result in a resetting of the adipo–insular axis leading to adiposity during childhood, a hypothesis that requires further testing.

Other effects

Recent epidemiological evidence suggests that fetal life influences the risk of CVD later in life (55, 56). Animal studies have shown that diabetes can induce cardiovascular dysfunction in adult offspring (57). Few human studies have examined cardiovascular risk factors in offspring of diabetic pregnancies. By 10–14 years, offspring of diabetic pregnancies enrolled in the Diabetes in Pregnancy follow-up study at Northwestern University had significantly higher systolic and mean arterial blood pressure than offspring of non-diabetic pregnancies (23). Higher concentrations of markers of endothelial dysfunction (ICAM-1, VCAM-1, E-selectin), as well as increased cholesterol-to-HDL ratio, were reported among offspring of mothers with T1DM compared with offspring of non-diabetic pregnancies, independent of current BMI (55). Recently, the Pima Indian investigators have shown that, independent of adiposity, 7–11-year-old offspring exposed to maternal diabetes

during pregnancy have significantly higher systolic blood pressure than offspring of mothers who did not develop T2DM until after the index pregnancy (58). These data suggest that *in utero* exposure to diabetes confers risks for the development of CVD later in life, independent of adiposity, and may be in addition to genetic predisposition to diabetes or CVD (55).

Intrauterine Growth Restraint

Human epidemiological studies over the last 15 years have provided strong evidence of an inverse association between size at birth and the development of adult glucose intolerance, T2DM, and the IR syndrome (59–61). Subsequently, over 30 studies worldwide have confirmed different aspects of this research (5, 7). The association has been explained as representing long-term effects of nutritional deprivation *in utero* on fetal growth, development of the endo1crine pancreas, and future risk for IR and T2DM (the "thrifty phenotype hypothesis") (7), or as following from pleiotropic effects of genes influencing both fetal growth and susceptibility to IR/T2DM (the "thrifty fetal genotype hypothesis") (62). Direct evidence that poor maternal nutrition can have detrimental consequences for adult glucose tolerance came from a study of adults who were *in utero* during the Dutch Famine towards the end of World War II. Offspring of these pregnancies were found to have reduced glucose tolerance, an effect most marked in those who were in the last trimester of pregnancy during the Famine (63). More recently, evidence for adverse consequences of a poor maternal environment has come from studies of glucose tolerance in offspring of mothers who smoked during pregnancy. Smoking in pregnancy, long recognized as a cause of reduced birth weight, increased the amount of T2DM in the offspring (64).

An important challenge of the thrifty phenotype hypothesis is that the exact nature and number of intrauterine insults that translate into intrauterine growth restraint in contemporary societies are not known, and maternal diet during pregnancy does not seem to completely account for offspring's size at birth (65). In addition, fetal nutrition, which is a function of maternal body size and nutritional status, uterine perfusion, placental function, and fetal metabolism, is likely to be more important than maternal diet *per se* in determining future chronic disease risk (66). Moreover, the association between birth weight and components of the IR syndrome in adult life was greatly enhanced by the presence of adult obesity (67), suggesting an interaction between *in utero* insults and postnatal growth trajectory, on later outcomes.

Intrauterine growth restraint and catch-up growth

A recent explanation for the increased risk of future development of the IR syndrome among low-birth-weight individuals is that of an interaction between fetal growth restraint and early childhood growth (65). The highest risk of future chronic diseases seems to be among people who were small at birth and became overweight during

childhood and early adulthood. The "fetal origins hypothesis" suggests that the interaction between small size at birth and obesity later in life reflects an integrated pathogenesis. From this perspective it was inferred that early postnatal "catch-up growth" modifies intrauterine influences. In the Avon Longitudinal Study of Parents and Children (ALSPAC) (65), Caucasian infants who showed catch-up growth during the first 1 to 2 years of life were smaller and thinner at birth but were larger and fatter than other children at 5 years. In this contemporary birth cohort, 30.7% of children showed catch-up growth, defined as a gain in SD score for weight between zero and 2 years, greater than the width of any percentile band on standard growth charts (greater than 0.67 SD scores). These children were thinner at birth than other children, indicating that their fetal growth had been restrained.

Effects on childhood growth and risk for obesity

Among 5210 Finnish individuals who were born between 1924 and 1933, BMI at age 7 (obtained from school health records) was a strong risk factor for adult obesity, and the association was only partly explained by maternal BMI (68). Moreover, the growth of those individuals, who later developed obesity, was faster in height, weight, and BMI from birth to age 7. Consistent with previous findings (69, 70), the relationship between birth weight and later obesity tended to be "J" shaped. It is possible, as the Finnish authors suggest, that babies who have a low birth weight lack muscle, since muscle tissue mostly develops during late gestation. They will have a disproportionate fat-to-lean mass if they become overweight, and this will further increase their risk to develop the metabolic syndrome later.

Effects on glucose–insulin metabolism

Very little is known about the effects of intrauterine growth restraint, as a marker of yet unknown, intrauterine exposure(s), and its interaction with early growth patterns on estimated insulin resistance and insulin secretion among children. A summary of key studies that have examined the relationship between IR and birth size among children is given in Table 4.1. Most of the available data indicate that, in general, intrauterine growth restraint is associated with markers of insulin resistance, *after adjusting for attained BMI* (Table 4.1). However, in 9–12-year-old British children, birth weight was negatively associated with 30-min blood glucose, independent of gestation or subsequent growth (71). In contrast, plasma insulin concentrations were more strongly associated with the pattern of childhood weight gain than with growth *in utero*: higher insulin concentrations were seen in children with the greatest increase in weight SDS between 18 months and current follow-up. In the ALSPAC cohort, the association between low birth weight and increased IR at 8 years of age was accounted for by the rapid growth in the first two years of life (72). Among 4-year-old Asian Indian children, low birth weight was associated

Table 4.1 Studies relating measures of insulin resistance to size at birth

Population	*N*	Age (years)	Outcome	Measurement	Relationship
Salisbury children (88)	250	7	Fasting insulin	Ponderal index	Inverse association
Pune Children (73, 74)	379	4	30-min insulin	Birth weight	Inverse association
		8	Fasting, 30-min, 2-h insulin	Birth weight	Inverse association with fasting and 30-min insulin;* No association with 2-h insulin
U.K. children (89)	1138	10–11	Fasting, 30-min insulin	Birth weight	Inverse association*
NHW and AA U.S. adolescents (90)	296	15	Fasting insulin, IR (euglycemic clamp)	Birth weight	Inverse association with fasting insulin;* No association with insulin sensitivity
AA U.S. youth (75)	53	4–14	Fasting insulin, visceral fat, IR (FSIGT)	Birth weight	Inverse association
U.S. Pima Indians (69)	2272	5–29	Fasting, 2-h insulin, HOMA-IR	Birth Weight	Inverse association*

IR = insulin resistance; FSIGT = frequently sampled intravenous glucose tolerance test; *adjusted for body mass index

with increased glucose and insulin concentrations (73) and with most of the IR syndrome components in healthy 8-year-old Indian children (74). In the United States, among Pima Indian children and young adults, birth weight was inversely associated with fasting and 2-h insulin levels, and with IR estimated from the homeostasis model, when adjusted for current weight and height (69). Another U.S. study tested the effects of low birth weight on components of the IR syndrome in 139 non-Hispanic white and African-American children aged 4–14 years, and showed that low birth weight was significantly associated with increased fasting insulin concentrations and visceral fat mass only among African-American children (75). However, early life growth was not measured, so the authors could not address the question of whether early life trajectory interacts with low birth weight in increasing measured or estimated IR during childhood.

Effects on other components of the IR syndrome

Among adult diseases associated with fetal growth retardation, hypertension is the most extensively studied. Approximately 80 studies involving more than 444,000 subjects support the inverse relationship between low birth weight and higher systolic

blood pressure in adults (76). In youth, although the weight of the evidence seems to favor an inverse relationship, the data are not uniform. As with previously discussed outcomes, the vast majority of the data come from studies in European Caucasians and Asians. Among 17-year-old Israeli youth, no correlation was found between birth weigh and blood pressure, but a positive correlation between blood pressure and BMI at age 17 existed (77). It was suggested, as with previously discussed outcomes, that current body size is a much more important determinant of blood pressure in children than size at birth (77, 78). Data also suggested that race or gender may modify the association between birth weight and childhood blood pressure (79). Very strong evidence for an association between intrauterine growth restraint and blood pressure in childhood comes from the ALSPAC study (80), which showed a graded inverse relation between birth weight and systolic (−1.91 mmHg/kg, $p < 0.0001$) and diastolic (−1.42 mmHg/kg, $p < 0.0001$) blood pressure among 3-year-old Caucasian children, after adjustment for current BMI. Although birth length, head circumference, and ponderal index at birth were also inversely related to blood pressure, these relationships disappeared after adjustment for birth weight. The strength of the association was not strongly influenced by maternal body size or by the children's growth pattern in the first year of life.

The association of fetal growth retardation with dyslipidemia is less well established in adults and there are almost no data in children. Dyslipidemia is part of the metabolic syndrome, which seems to be more common in adults with low birth weight, and may be at least partly a result of IR (81). Increased BMI, central adiposity, IR, hypertension, and dyslipidemia in childhood were shown to track during adulthood and predict later T2DM and CVD (82).

Intrauterine growth restraint and insulin-like growth factors

The mechanisms, by which intrauterine growth restraint/postnatal catch-up growth predispose to chronic diseases later in life, are also hypothetical. It has been suggested that various metabolic/endocrine mechanisms in the fetus may respond to undernutrition to ensure fetal survival. The development of IR is consistent with growth retardation in response to poor placental nutrition and it may represent a mechanism to optimize fetal survival (83). IR may be exacerbated by postnatal catch-up growth with its associated increased central fat deposition (65). In this context, the effects of insulin-like growth factors (IGFs) may be important. Both leptin and IGF-1 measured in the umbilical cord positively correlate with birth weight (84). The regulation of fetal IGF-1 *in utero* is primarily influenced by placental glucose transfer, which regulates fetal insulin release. It has been hypothesized that, in the face of an intrauterine environment that cannot offer the fetus optimal conditions for growth, the fetus may respond by reducing IGF-1 production, in order to ensure survival (85). It has also been shown that intrauterine growth retardation is associated with later resistance to insulin, IGF-1, and growth hormone (63). Higher rates of postnatal weight gain have also been related to lower satiety in low birth weight infants, as assessed by the volume of milk intake among bottle-fed infants (86), while in the

ALSPAC population-based study, leptin levels at birth were inversely related to rates of infant growth (87).

Summary

It appears that increased risks for adiposity, insulin resistance, and related metabolic consequences occur among children born at both ends of the birth weight spectrum: generalized obesity with exposure to maternal hyperglycemia (also resulting in higher birth weight) and increased visceral adiposity and its metabolic consequences at lower birth weights. Future research should help to disentangle the effects of selected fetal exposures on childhood risk for obesity and associated metabolic conditions, and to understand whether these exposures have direct biological influences or whether they are mediated through later lifestyle choices. Such studies could ultimately lead to the development of strategies for early-life prevention of future chronic disorders.

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Chapter 5
Early Childhood Contributions to Insulin Resistance

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Keywords low birth weight, catch-up growth, insulin resistance, insulin-like growth factor-I, disposition index

Introduction

It is more than 15 years since Barker and Hales first reported a relationship between size at birth and adult risk for the development of impaired glucose tolerance (IGT), type 2 diabetes (T2D) and cardiovascular disease (CVD) (1–3). These observations have now been replicated in many populations, and do not appear to be confounded by socioeconomic and environmental factors. However, the data have largely been gathered through the retrospective study of birth records and the pathophysiological mechanisms underlying these associations remain unclear.

The association between low birth weight and increased risk for T2D, in particular, is often only evident after allowing for larger current body size, implying a dependence on the transition from smaller size at birth to overweight or obesity in adulthood (4). In the contemporary birth cohort of the Avon Longitudinal Study of Parents and Children (ALSPAC), smaller birth size followed by rapid early postnatal weight gain was a risk factor for increased body fat mass and central fat distribution at 5 years of age (5). The link between T2D risk and the transition from smaller size at birth to larger childhood size has been attributed to the development of insulin resistance (6–9). Consistent with this, larger body mass index (BMI), increased waist circumference, and insulin resistance in children aged 8 years were predicted by more rapid weight gain in the first three postnatal years (10).

This chapter reviews what has been learnt about how pathways from smaller size at birth through rapid infancy weight gain lead to future disease risk.

P.S. Zeitler, K.J. Nadeau (eds.) *Insulin Resistance: Childhood Precursors and Adult Disease*,

Size at Birth and Risk for Adult Disease

Over the last decade, the link between small size at birth and risk for disease in adulthood has been established from population-based studies, where archival birth records were traced and analyzed with respect to long-term outcomes. Low birth weight was associated with an increased risk for CVD (1, 11–14), T2D, and hypertension in adult life (3, 11, 15–21). These associations are not confined to differences between the smallest and other infants, but a continuum of varying risk is observed throughout the whole range of birth weights. For example, the original studies in men born in Hertfordshire between 1911 and 1930 indicated that those with above-average birth weights had 24% lower standardized mortality rates from coronary heart disease compared to those with average birth weights (1). In some populations, the association of birth weight and adult disease risk appears to be U-shaped, with babies born the heaviest also having increased long-term risk for disease (22, 23), perhaps reflecting the risk associated with maternal gestational diabetes (24). Eriksson et al. studied a large Finnish birth cohort and described size at birth and early postnatal growth patterns for 290 adults with T2D (25). Sixty-six percent of T2D subjects were born smaller than average, showed rapid weight gain during the first two years of life, and continued to gain weight rapidly. Thirty-four percent of T2D subjects had relatively large birth weights, possibly due to gestational diabetes, and demonstrated initial losses in weight and length centile position. However, from the age of 2 years, these children gained in weight centile progressively and became obese.

Epidemiological studies indicate that size at birth may influence early weight gain, fat distribution, and long-term risk for obesity. In a study of 300,000 19-year-old men exposed to the Dutch famine between 1944 and 1945 (26), there was a nearly twofold increase in obesity risk in those subjects whose mothers were exposed to famine during the first trimester of pregnancy. Gale et al. (27) showed that, among 70- to 75-year-old men studied by dual energy X-ray absorptiometry (DEXA), low birth weight was associated with reduced lean tissue mass and greater body fat relative to current weight. Thus, the predisposition to adult disease conferred by low birth weight may be related to excess fat deposition, particularly central fat, and hence the development of insulin resistance. One study (28), using the gold standard hyperinsulinemic-euglycemic clamp assessment of insulin sensitivity in 70-year-old men, showed that the association between low birth weight and insulin resistance was seen largely in the highest BMI tertile group. It therefore appears to be the transition from relatively low birth weight to larger postnatal body size that confers disease risk. Furthermore, with the increasing abundance of nutrition and rising rates of obesity even in childhood, such a transition may now be occurring at much younger ages (29, 30).

Prenatal Exposures

Critical windows of prenatal and early postnatal life exposures proposed by Widdowson (31) appear to be important in determining the long-term disease risk. In humans, in addition to fetal genes, the maternal uterine environment is an important

determinant of size at birth (5). For example, the growth of first-born babies appears to be restrained, as they are smaller at birth, and then show rapid postnatal catch-up weight gain (32). In these first-borns, birth weight correlations with maternal and grand-maternal birth weights are particularly strong (33, 34). The nature of this maternal inheritance of birth weight is unclear. Associations between birth weight and common genetic variation in mitochondrial genes, which are inherited only from the mother, and imprinted genes, where only the maternal copy is expressed, have been described (35, 36). More recently, attention has turned to epigenetic mechanisms whereby the maternal uterine environment could permanently alter methylation of the genome and therefore later gene expression (37). Curiously, low birth weight in the mother is also associated with an increased risk of gestational diabetes in the offspring (38). This observation illustrates the paradox of association between both low and high birth weights and increased risks for T2D. In the Cambridge Birth Cohort, mothers of first-born babies had higher blood glucose levels than others who were having their second or third baby (36), and it is possible that the mechanisms for maternal restraint of fetal growth could also, in genetically susceptible individuals, lead to gestational diabetes.

The mechanisms underlying programming of disease risk *in utero* are likely to be complex and probably involve an interaction between fetal genes and the maternal uterine environment. It is becoming clearer that these prenatal interactions increase the subsequent risk for the development of obesity and insulin resistance, and therefore disease in adult life.

Consequences of Intrauterine Growth Retardation

Prenatal growth restraint may influence patterns of postnatal weight gain and the subsequent risk for the development of obesity. In the ALSPAC cohort, about 25% of infants showed postnatal rapid catch-up weight gain (they crossed centiles upwards over the first 6 to 12 months) and around 25% exhibited catch-down in weight relative to their birth centile (5). The remaining 50% of infants grew steadily along the weight centile on which they were born. It has been debated whether this realignment of growth patterns represents true “catch-up” and “catch-down” growth. Observations in the ALSPAC cohort indicate that these postnatal growth patterns are clearly related to prenatal exposures on fetal growth such as parity, maternal smoking, and maternal birth weight, indicating that they may reflect reversal of the effects of restraint or enhancement of fetal growth (5).

Catch-up weight gain seems to be driven by satiety, as it can be predicted from cord blood leptin and ghrelin levels (39, 40), and is associated with increased levels of nutrient intake at age 4 months (41). Catch-up in height also occurs in these infants, but this is generally completed by the age of 6 to 12 months, and growth then continues along a centile appropriate for mid-parental height (42). In contrast, the rapid weight gain may continue and, in the ALSPAC cohort, the early catch-up group had the greatest BMI, percentage body fat, and fat mass at age 5 years when compared with the no-change or catch-down groups (5). In addition, catch-up

infants had an increased waist circumference at 5 years, which may be critical with regard to future metabolic risk.

The early excess weight gain in catch-up infants from the ALSPAC cohort persisted up to the age of 8 years, and similar consequences of rapid weight gain in the first few months of life were seen in large cohort studies in the U.S. and Seychelles island group (43, 44). In the Stockholm Weight Development Study, a faster weight gain observed during the first 6 months of life predicted a greater percentage of body fat at age 17 years, independent of childhood weight gain, maternal size, and social factors (45). Early weight gain may also influence the distribution of body fat. In the ALSPAC study (5), children who showed early postnatal catch-up had the largest waist circumference at age 5 years. In other populations, the transition from low birth weight to normal or greater BMI during childhood has been associated with alterations in body composition and increased central fat deposition in children and adults (46–49). In the National Health and Nutrition Examination Survey III, 1988 to 1994 (50), children born small for gestational age showed reduced lean tissue and a higher percentage of body fat. Studies from Australia have also reported an association between low birth weight, higher current weight, and increased central fat deposition (51).

Thus, in contemporary populations, relatively low birth size followed by rapid early postnatal weight gain appears to be a risk factor for later obesity and central fat deposition. Such an association could be influenced by feeding practice, and recent studies suggest that breast milk, the type of formula milk used, or other infant nutritional variations could influence not only obesity risk but also other cardiovascular risk factors (52, 53).

Insulin Resistance

Central adiposity and accumulation of visceral fat, in particular, are important risk factors for the development of insulin resistance (51). In a recent study of small for gestational age (SGA) versus appropriate for gestational age (AGA) infants, Ibanez et al. described that an accretion of excess central fat in SGA infants occurred as early as 2 to 4 years (54). Garnett et al. showed that for each tertile of weight at 8 years, infants with low birth weight had the greatest percentage of abdominal fat (51). In the ALSPAC cohort, Ong et al. observed that catch-up infants were the most insulin resistant at age 8 years (10), and it was the overweight children with the lowest birth weight who were the most insulin resistant, but the effect of size at birth was only evident in those in the highest tertile of weight (Fig. 5.1).

Insulin resistance may develop during early postnatal life. In a recent case-controlled study in Chile, infants born SGA had lower insulin and glucose levels at age 48 h (55), which may be consistent with the finding of initially increased insulin sensitivity in animal models where there has been prior intrauterine growth retardation (56). However, after postnatal catch-up weight gain, these SGA infants

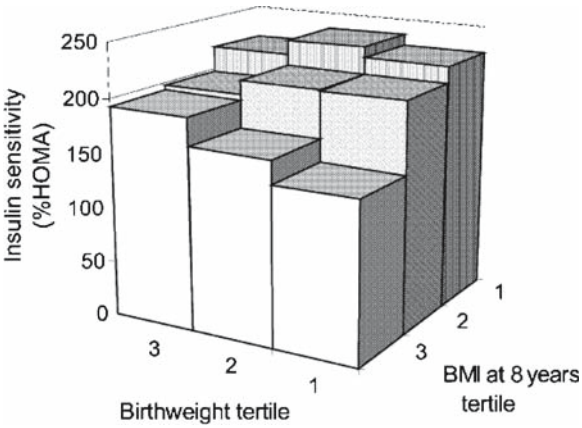

Fig. 5.1 Fasting insulin sensitivity (HOMA) at 8 years of age by tertiles of birth weight and current BMI. There was interaction between birth weight and current BMI on insulin sensitivity at 8 years (p-interaction <0.05), such that lower birth weight was related to lower insulin sensitivity only among children with the highest BMI at age 8 years (front row, p-trend = 0.006). Reproduced from Ong et al. (2004) with permission

had higher fasting insulin levels at ages 1 and 3 years, indicative of insulin resistance, even though they were still lighter than normal birth weight controls (57, 58).

Several studies have described insulin resistance in children and adults with a history of low birth weight. In a large French study (59), 20-year-old adults with birth weights below the third centile had higher fasting insulin and higher post-oral glucose insulin levels compared with normal birth weight controls. In a subset of that study, SGA subjects had lower insulin-stimulated glucose uptake and a lesser degree of free fatty acid suppression during the hyperinsulinemic euglycemic clamp, findings that confirm relative insulin resistance, and these differences were not entirely explained by body size or body fat mass (60).

The association between low birth weight and subsequent insulin resistance has been commonly attributed to poor fetal growth, and gestational age has been considered a confounding rather than a contributing factor. However, this conclusion needs to be reconsidered in light of data from New Zealand suggesting that premature babies also have higher postnatal insulin resistance, even when catch-up weight gain has been slow (61). A recent study from Finland showed that young adults born with very low birth weight (<1500 g) are more insulin resistant and have more impaired glucose tolerance than normal birth weight controls. Further analyses showed that low birth weight for gestational age had a greater impact on these long-term outcomes than simple prematurity alone (62). Whether gestational age itself is important or whether these observations simply reflect the fact that all preterm infants may have experienced relative growth retardation is yet to be determined.

Risk for the Metabolic Syndrome and T2D

Contemporary birth cohorts have only limited follow-up data, and the exploration of links between size at birth and risk for CVD have depended on surrogate end points. Nevertheless, there is data to indicate that low birth weight followed by postnatal catch-up growth leads to increased risk for dyslipidemia (63, 64), abnormalities in adipocytokine profile (65), and vascular reactivity (66). These observations reflect the close link between CVD risk factors, obesity, and the development of insulin resistance (67).

Although insulin resistance is an important risk factor for T2D, insulin resistance *per se* only leads to diabetes if there is failure of beta cell compensation. The relationship between insulin resistance and insulin secretion is parabolic, and beta cell capacity is best described by the product of the two; the "disposition index" (68). In ALSPAC, the disposition index was assessed at age 8 years in over 800 children using a short oral glucose tolerance test with measurements of glucose and insulin at 0 and 30 min, where insulin secretion was estimated by calculating the insulinogenic index (69), and Homeostasis Model Assessment (HOMA) (70) gave an estimate of insulin sensitivity. Lower disposition index was associated with lower ponderal index at birth, but not with the rate of postnatal weight gain (10). It was also closely related to height, mid-parental height, and insulin-like growth factor-I (IGF-I) levels; the children showing the least gains in postnatal height and with the lowest IGF-I levels had the lowest disposition index (10). Similar data have been reported from a Chilean cohort of SGA and AGA infants studied at a much earlier age (71). The difference in height gain between children in the highest and lowest tertiles of insulin secretion adjusted for sensitivity and IGF-I levels at 8 years is striking. The children with relatively poor insulin secretion aged 8 years show a pronounced loss in height standard deviation score (SDS) (Fig. 5.2) and reduced levels of IGF-I between ages 6 months to 1 year. This is a critical period for determining height trajectory (31), which, in early infancy, is regulated by insulin and IGF-I (41, 72, 73).

Thus, following prenatal growth restraint, catch-up growth driven by reduced satiety can lead to insulin resistance and visceral fat accumulation, but height gain and IGF-I levels may be more important markers of beta cell mass and the subsequent risk for the development of T2D. In ALSPAC, children with the least height gain by 8 years have the lowest insulin secretion, despite being relatively insulin sensitive. Indeed, the insulin sensitivity may be an adaptive response to poor insulin secretion.

However, the children who probably give the greatest concern are those who remain short and fat, with the lowest insulin sensitivity. Although they showed compensatory hyperinsulinemia, their insulin secretion was less than that seen in the other subjects (unpublished). The same relationship between height, IGF-I levels, insulin secretion, and risk for T2D has been observed in adults (74). In adults with normal glucose tolerance, low IGF-I levels were associated with short stature and IGT and T2D risk (75). Rapid weight gain, abdominal obesity, and the development of insulin resistance may be the environmental exposure,

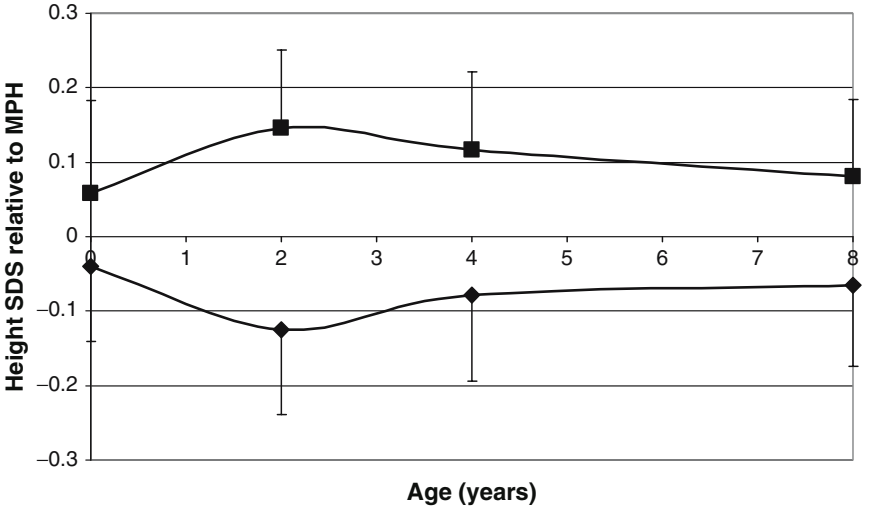

Fig. 5.2 Height SDS from birth to age 8 years by extreme tertiles of insulin secretion adjusted for insulin sensitivity (disposition index). Data are mean ±1 SE. ◆ = lowest tertile; ■ = highest tertile. Repeated measures analysis showed significant differences in height SDS over time ($p = 0.03$)

but prenatal environmental, genetic, and epigenetic determinants of beta cell mass may be the most important determinants of T2D risk.

Conclusion

Understanding the mechanisms underlying links between size at birth, postnatal growth, and risk for T2D has important implications for public health. In countries such as India, where nutrition has recently improved, particularly with population migration from rural to urban environments or emigration, babies born small are at high risk for developing T2D (76, 77). With regard to contemporary Western countries, the risks associated with low birth weight due to poor maternal nutrition during pregnancy are much lower (78–80), and conversely the risks related to increasing rates of maternal obesity and gestational diabetes are of greater concern (24, 29, 30). A recent study of women in Eastern Europe showed that an increase in maternal pregnancy weight gain is one of the first responses to socioeconomic improvement (81). Data from the Pima Indians demonstrated that even borderline increases in maternal blood glucose levels during pregnancy may increase the risk of T2D in the offspring (82).

The complex interaction between the maternal uterine environment and fetal genes has evolved over many thousands of years to optimize maternal and fetal

survival (83, 84). The recent changes in the nutritional status of mothers and offspring may not just be associated with obesity, but could also alter the balance of risk for adult disease such as T2D.

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Part II
Pathophysiology

Chapter 6
Molecular Mechanisms of Insulin Resistance

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Keywords insulin resistance, insulin action, IRS-1, PI3-kinase

Even though insulin resistance has emerged as an enormous health care problem, encompassing the fields of obesity, diabetes, hypertension, and cardiovascular diseases (1,2), its molecular mechanism remains incompletely understood. Clinically, the term *insulin resistance* implies that higher than normal concentrations of insulin are required to maintain normoglycemia. In other words, insulin-resistant humans and animals develop compensatory hyperinsulinemia in order to ensure normal utilization of glucose by the insulin target tissues (3). Physiologically, insulin is released from the pancreatic ß-cells post-prandially in order to maintain euglycemia. Insulin promotes glucose uptake in skeletal muscle and fat by stimulating translocation of glucose transporter 4 (GLUT 4) from the cytosol to the plasma membrane where it facilitates glucose transport (4,5). Concomitantly, insulin stimulates intracellular utilization of glucose by many other tissues as well. Post-absorptively (i.e. in the fasting state), the main physiological task of insulin is to suppress glucose production by the liver. Therefore, if either of these main aspects of insulin action is impaired, one can encounter insulin resistance either at the level of skeletal muscle and fat or hepatic insulin resistance, both of which contribute to the total body insulin resistance.

On a cellular level, insulin plays an important role not only in the carbohydrate metabolism, but also in protein and lipid synthesis, ion fluxes, cell growth and differentiation, inhibition of lipolysis, protein degradation, and apoptosis. A possibility that not all aspects of insulin action are affected equally by insulin resistance gave rise to the concept of "selective insulin resistance". It also became apparent that many "metabolic" aspects of insulin action are mediated via stimulation of a distinct intracellular signaling pathway from the pathway involved in activation of the "mitogenic" aspects of insulin action (6–15). Thus, on a cellular level, the term "insulin resistance" defines an inadequate strength of insulin signaling from the insulin receptor downstream to the final substrates of insulin action involved in multiple metabolic and mitogenic aspects of cellular function (16,17).

Insulin action is initiated by an interaction of insulin with its cell surface receptor (18,19). The insulin receptor is a heterotetrameric protein that consists of two

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extracellular alpha subunits and two transmembrane beta subunits connected by disulfide bridges (20–22). Insulin binding to the extracellular alpha subunit induces conformational changes of the insulin receptor that activate the tyrosine kinase (TK) domain of the intracellular portion of the beta subunit (23–26). Once the TK of insulin receptors is activated, it promotes autophosphorylation of the beta subunit itself, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162 and Tyr-1163) is required for amplification of the kinase activity (27,28). Activation of the TK of the insulin receptor also leads to a rapid phosphorylation of the so-called "docking proteins", such as insulin receptor substrate (IRS)-1, 2, 3, and 4, and several Shc (Src-homology collagen) proteins (52-, 46-, and 64-kD isoforms) (29,30), which, in turn, attract multiple intracellular signaling intermediates.

Initial attempts to unravel the molecular mechanism of insulin resistance have strongly suggested that a defect responsible for insulin resistance in most patients lies at the post-receptor level of insulin signaling (31–33). Thus, numerous studies have demonstrated that the number and function (tyrosine kinase activity) of insulin receptors are either normal or only slightly reduced in patients and experimental animals with insulin resistance, insufficient to account for a substantial reduction in insulin action.

Furthermore, studies in the laboratory of Petersen and Shulman (34), using magnetic resonance spectroscopy *in vivo* to measure intracellular concentrations of naturally occurring isotopes (^{1}H, ^{13}C, and ^{31}P), indicated that insulin resistance in patients with type 2 diabetes and offspring of patients with type 2 diabetes is attributable mostly to a defect in insulin-stimulated glucose transport into skeletal muscle. Thus, the question as to why insulin-stimulated glucose uptake is defective in insulin resistance remains a subject of intense investigation.

The IRS and Shc proteins play an important regulatory role in the insulin signaling cascade, as in their phosphorylated form they become points of anchoring for intracellular proteins containing Src-homology-2 (SH-2) domains (18,19). Whereas interaction of IRS and Shc proteins with the intracellular domain of the insulin receptor constitutes the first step in dispersing the directions of insulin signaling intracellularly, their ability to attract multiple signaling intermediates to their own phosphorylated domains further partitions insulin signaling downstream, thereby accounting for the multitude of insulin's biological effects (18,19).

Most, if not all, of the metabolic and anti-apoptotic effects of insulin are mediated by the signaling pathway involving IRS proteins, phosphorylation and activation of phosphatidylinositol 3-kinase (PI 3-kinase), Akt (also known as protein kinase B or PKB), mTOR (molecular target of rapamycin), and p70 S6 kinase (6–9). Activation of PI 3-kinase, Akt and atypical protein kinase C (PKC) via the phosphoinositide-dependent protein kinase (PDK) (10) appears to be critical in the mechanism of insulin action on GLUT-4 translocation and glucose transport. In contrast, non-metabolic, proliferative, and mitogenic effects of insulin are mediated largely via the activation of Ras (mostly through Shc and to a lesser degree through IRS proteins), Raf, and mitogen-activated protein (MAP) kinases Erk 1 and Erk 2 (11–15).

Activation of PI 3-kinase results in the generation of phosphatidylinositol triphosphate, PIP_3, or phosphoinositide 3,4,5-P_3, which in turn phosphorylates a serine kinase Akt (35). Post-translationally, Akt binds to heat shock protein 90,

protecting the inactive Akt from proteasomal degradation (36). Akt is then recruited to the cell membrane through PIP_3 (35,37). In addition to Akt, PIP_3 also recruits PDK1, which phosphorylates and activates Akt. Generation of PIP_3 is negatively regulated by phosphatase and tensin homolog (PTEN) that prevents activation of Akt by dephosphorylating PIP_3. PTEN deletion is the most common mechanism of hyperactivation of Akt in human malignancy (38).

Subsequent studies in insulin-resistant animal models and humans have consistently demonstrated a reduced strength of insulin signaling via the IRS-1/PI 3-kinase pathway (39–41), resulting in diminished glucose uptake and utilization in insulin target tissues. However, the nature of the culprit that initiates and sustains impaired insulin signal transduction along the IRS-1/PI 3-kinase pathway is still largely enigmatic. Two separate, but likely complementary mechanisms have recently emerged as a potential explanation for the reduced strength of the IRS-1/PI 3-kinase signaling pathway.

Serine phosphorylation of IRS-1

First, it became apparent that serine phosphorylation of IRS proteins can reduce the ability of IRS proteins to attract PI 3-kinase, thereby minimizing its activation (42–48), and can also lead to an accelerated degradation of IRS-1 protein (49). Thus, in contrast to a signal promoting tyrosine phosphorylation, excessive serine phosphorylation of IRS proteins could become detrimental for normal conductance of the metabolic insulin signaling downstream, causing insulin resistance. Serine phosphorylation of IRS proteins can occur in response to a number of intracellular serine kinases (Table 6.1).

A cellular nutrient sensor, mTOR, has been identified as a critical element integrating cellular metabolism with growth factor signaling (50–53). In response to insulin and amino acids, mTOR, which is a serine/threonine kinase, phosphorylates and modulates the activities of p70 S6 kinase (S6K1 kinase) and an inhibitor of translational initiation, eIF-4E binding protein (eIF-4EBP) (54–56). mTOR interacts with two scaffolding proteins, Raptor (57,58) and Rictor (59–61). The Raptor/mTOR complex is rapamycin sensitive and regulates growth via S6K1 and eIF-4EBP (57,58). The Rictor/mTOR complex is insensitive to rapamycin and

Table 6.1 Causes of IRS-1 serine phosphorylation

(a) Mtor – p70S6 kinase-amino acids, hyperinsulinemia, TSC1-2 depletion, nutrition (97–100)
(b) JNK – stress, hyperlipidemia, inflammation (70–73,101)
(c) IKK – inflammation (79–82)
(d) TNF-α – obesity, inflammation (85–89)
(e) Mitochondrial dysfunction (90–92)
(f) PKCθ – hyperglycemia, diacylglycerol, inflammation (73, 93–96)

regulates cellular proliferation via Akt (62), PKCα (61), and small molecular weight GTPases (59). While insulin activates mTOR and S6K1 kinase via the IRS-1/PI 3-kinase/Akt pathway (63,64), amino acids seem to exert their effect through a direct influence of mTOR (52, 65,66). In any event, activation of mTOR and S6K1 kinase causes serine phosphorylation of IRS-1, with a subsequent decline in the IRS-1-associated PI 3-kinase activity (Fig. 6.1A). In contrast to wild-type

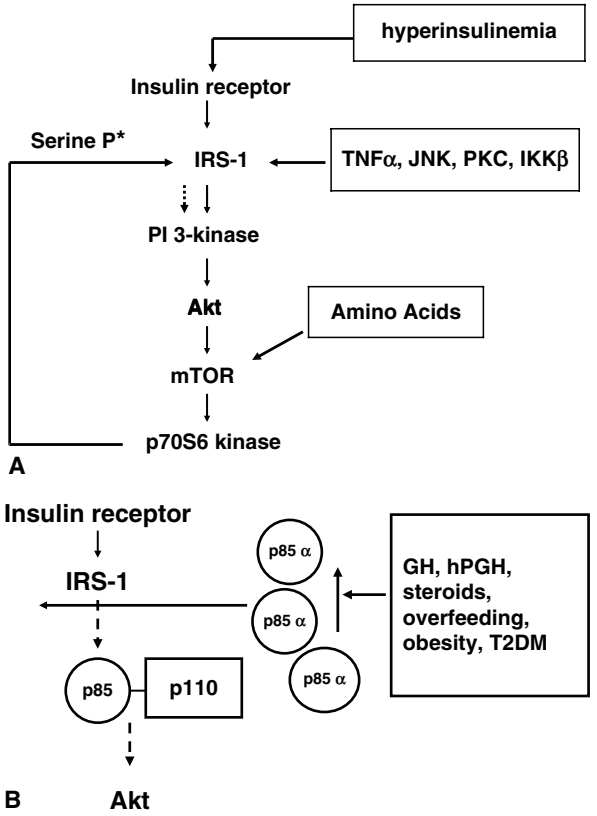

Fig. 6.1 Inhibition of metabolic insulin signaling**.** IRS-1 is phosphorylated by the tyrosine kinase of the insulin receptor in response to insulin binding. Protein/lipid kinase, PI 3-kinase binds to the specific MYMX motifs of IRS-1, containing phosphorylated tyrosine residues. PI 3-kinase is then activated and initiates a downstream cascade of events leading to the phosphorylation and activation of Akt, mTOR, and p70S6 kinase. Activation of Akt appears to be important for glucose transport, while activation of mTOR and p70S6 kinase participates in the process of protein synthesis. **A.** Hyperactivation of mTOR by amino acids, Akt, or hyperinsulinemia results in serine phosphorylation of IRS-1 by p70S6 kinase with a subsequent decrease in the strength of the IRS-1/PI 3-kinase signaling. In addition, serine phosphorylation of IRS-1 can be promoted by JNK, PKC, IKKβ, and TNFα. **B.** Increased expression of p85α monomer competes with and displaces the p85–p110 heterodimer from the IRS-1 binding sites. The resultant decrease in association of p110 with IRS-1 diminishes PI 3-kinase activity and the downstream effects of this kinase. Steroids, GH, hPGH, a short-term overfeeding, obesity, and type 2 diabetes have been shown to increase p85α expression (see text for details and references)

littermates, transgenic mice lacking S6K1 kinase (S6K1-deficient mice) displayed a strong resistance to age- and diet-induced obesity and insulin resistance (45). Moreover, because wild-type mice on a high fat diet demonstrated significantly elevated S6K1 kinase activity and serine phosphorylation of IRS-1, it has been suggested that under conditions of nutrient saturation S6K1 kinase may negatively regulate insulin signaling and sensitivity (45,67,68).

In addition to the inhibitory phosphorylation of IRS-1 on Ser 307 by S6K1, mTOR can directly phosphorylate IRS-1 on Ser 636/639 (human isoform) (69). The mTOR-S6K1-mediated serine phosphorylation of IRS-1 can act as a homeostatic negative feedback loop in response to nutrients, and, possibly, hyperglycemia. In a state of nutritional excess, such as obesity and type 2 diabetes, compensatory hyperinsulinemia may synergistically hyperactivate the mTOR/S6K1 pathway, leading to serine phosphorylation of IRS-1, its degradation, and further decline in PI 3-kinase activity.

Because insulin resistance can be induced by mechanisms other than nutritional excess, serine phosphorylation of IRS-1 has been examined under various circumstances. It appears that in addition to the mTOR-S6K1-dependent mechanism, various serine kinases, such as c-Jun amino terminal kinase (JNK), stress-activated protein kinases, tumor necrosis factor α (TNFα), and PKC, among others, can promote serine phosphorylation of IRS-1 (Table 6.1and Fig. 6.1A).

Activation of JNK by free fatty acids (FFAs), stress, and inflammation (70–73) has been shown to increase serine phosphorylation of IRS-1, with a resulting decline in the strength of insulin signaling along the metabolic pathway (74,75). Blocking JNK activation rescued the cellular and molecular defects induced by FFAs (71). Furthermore, JNK-1 knockout mice were found to be resistant to diet-induced obesity and insulin resistance (70). Treatment of cells with a specific JNK activator, anisomycin, was reported to elicit IRS-1 phosphorylation on Ser 307 (76). Increased serine 307 phosphorylation of IRS-1 has been found in the liver of the wild-type but not JNK-1-deficient mice (77).

Similarly, activation of the pro-inflammatory kinase that phosphorylates the inhibitor of NF-κB, inhibitor kappa B kinase β (IKKβ), has been shown to induce insulin resistance (78–80). In an unstimulated state, NF-κB dimers are restrained in the cytoplasm in association with inhibitory proteins IκBs. In response to pro-inflammatory stimuli, such as TNFα, IKKβ is activated and phosphorylates two serine residues of the IκB. Phosphorylated IκB is rapidly degraded by proteasomes, releasing NF-κB for translocation to the nucleus where it activates transcription of target genes. Inhibition of IKKβ with salicylates has been shown to prevent and reverse diet- and obesity-induced insulin resistance (81,82).

Activation of IKKβ in skeletal muscle is associated with impaired IRS-1/PI 3-kinase signaling (83). Furthermore, Kim et al. (84) have demonstrated that activation of IKKβ by hyperglycemia plays an important role in impaired insulin-stimulated nitric oxide production in endothelial cells. Overexpression of wild-type IKKβ recapitulated the deleterious effect of hyperglycemia on insulin-mediated activation of endothelial nitric oxide synthase (84). Taken together, available evidence implicates IKKβ in the pathogenesis of insulin resistance via a mechanism that involves impairment in the IRS-1/PI 3-kinase signaling pathway.

TNFα, an agent responsible for cachexia, has been shown to be increased in adipose tissue of obese, insulin-resistant humans and animals. Because removal of TNFα appeared to reverse insulin resistance in animal models, it has been suggested that TNFα plays an important role in the pathogenesis of insulin resistance in obesity (85–87). Furthermore, mice lacking TNFα function were protected from obesity-induced insulin resistance (88). More recently, TNFα has been shown to block insulin signaling by promoting serine phosphorylation of IRS-1 (89), with a resultant decline in IRS-1-associated PI 3-kinase activity.

Recently, a hypothesis that mitochondrial dysfunction or reduced mitochondrial content accompanied by a decreased mitochondrial fatty acid oxidation and accumulation of fatty acid acyl CoA and diacylglycerol can cause insulin resistance has gained substantial experimental support (90–92). The mechanism of insulin resistance in these cases has been suggested to involve activation of a novel PKC, which either by itself or via IKKβ or JNK-1 could lead to increased serine phosphorylation of IRS-1.

The pro-inflammatory novel PKCθ has been found to cause serine phosphorylation of IRS-1 (93,94), while PKCθ knockout mice have been shown to be protected from fat-induced insulin resistance (95). Increased activity of PKCθ along with increased activity of JNK has also been found in the skeletal muscle of obese and type 2 diabetic subjects (73,96), supporting a potential role of these serine kinases in the pathogenesis of insulin resistance.

Increased expression of p85α

A second molecular mechanism that can potentially lead to insulin resistance is a disruption in the balance between the amounts of the PI 3-kinase subunits (102). PI 3-kinase belongs to the class 1a 3-kinases (103) that exist as heterodimers, consisting of a regulatory subunit (p85), which is tightly associated with a catalytic subunit, p110. The regulatory subunit, p85, is encoded by at least three genes that generate highly homologous products. Two isoforms are termed p85α (PIK3R1) and p85β (products of the two genes). Three splice variants of p85α have been reported, including p85α itself, p55α and p50α. The third gene product is p55γ. The p85α, however, appears to be the most abundant isoform (103).

The main function of the class 1a 3-kinases is to produce phosphoinositide 3,4,5-P_3, one of the major signaling components of the cell. These kinases are obligate heterodimers because p110 catalytic subunits are unstable as monomers in mammalian cells (104). The p85 regulatory subunit stabilizes the p110 subunit (105–107) and maintains it in a low activity state (104). Activation of the p85–p110 heterodimer involves a conformational change disinhibiting p110. It appears that the *N*-terminal SH2 domain of the regulatory p85 subunit (nSH2) is the major regulator of p110 activity (108–110). The nSH2 domain of p85 inhibits p110 activity, and its interaction with a phosphopeptide disinhibits the p85-p110 heterodimer's activity (108–110).

Normally, the regulatory subunit exists in stoichiometric excess to the catalytic one, resulting in a pool of free p85 monomers not associated with the p110 catalytic subunit. Thus, there exists a balance between the free p85 monomer and the p85–p110 heterodimer, with the latter being responsible for the PI 3-kinase activity. Increases or decreases in expression of p85 shifts this balance in favor of either free p85 or p85–p110 complexes (111–114). Because the p85 monomer and the p85–p110 heterodimer compete for the same binding sites on the tyrosine-phosphorylated IRS proteins, an imbalance could cause either increased or decreased PI 3-kinase activity (Fig. 6.1B). This possibility has been recently supported by studies in insulin-resistant states induced by human placental growth hormone (115), obesity, type 2 diabetes (73), and by short-term overfeeding of lean non-diabetic women (116).

One of the first indications that an imbalance between the abundance of p85 and p110 can alter PI 3-kinase activity came from experiments with L-6 cultured skeletal muscle cells treated with dexamethasone (117). This treatment significantly reduced PI 3-kinase activity, despite an almost 4-fold increase in the expression of p85α (no change in p85β) and only a minimal increase in p110. The authors concluded that p85α competes with the p85–p110 heterodimer, thereby reducing PI 3-kinase activity (Table 6.2).

Subsequently, animals with a targeted disruption of *p85α* ($p85^{+/-}$ heterozygous mice) have been found to have a higher ratio of p85–p110 dimer to free p85, and to be more sensitive to insulin (102,118,119). In order to determine this ratio, the authors immunodepleted p110 and blotted both the immunoprecipitates and the supernatant with p85 antibody. The amounts of p85 in the p110 immunoprecipitates denote p85 bound to p110, while the amount of p85 in the supernatant represents free (excess) p85. The greater the ratio of bound to free, the greater the insulin sensitivity the mice display. The same group of authors then overexpressed p85a in cultured cells. This overexpression significantly inhibited the PI 3-kinase activity (113,114,120). Overexpression of p50α or p55α did not inhibit PI 3-kinase activity to the same extent. These experimental results were consistent with the competition hypothesis.

Recently, Barbour et al. (115,121) demonstrated that insulin resistance of pregnancy is likely due to increased expression of the skeletal muscle p85 in response to increasing concentrations of the human placental growth hormone (hPGH). Furthermore, women remaining insulin resistant post-partum have been found to

Table 6.2 Causes of imbalance between PI 3-kinase subunits

(a) Steroids (119)
(b) Growth hormone (GH) (121)
(c) Human placental (hPGH) (115,121)
(d) Short-term overfeeding (116)
(e) Obesity and diabetes (73)

display higher levels of p85 in the muscle (122). Thus, results reported in the literature support the hypothesis that the p85 monomer competes with a p85–p110 dimer and that the removal of the excess of p85 improves insulin sensitivity by allowing the remaining isoforms to bring p110 to its site of action.

Finally, in a small study of eight healthy lean women without a family history of diabetes, Cornier et al. (116) were able to show that three days of overfeeding (50% above usual caloric intake) led to a significant increase in the expression of p85α and the ratio of p85α to p110, along with a decline in insulin sensitivity. Within this experimental time frame, overfeeding did not cause any change in serine phosphorylation of either IRS-1 or S6K1 (116), suggesting that increased expression of p85α may be an early molecular step in the pathogenesis of nutritionally induced insulin resistance.

Summary

There have been substantial strides made in our understanding of the genesis of insulin resistance. A number of serine kinases that could phosphorylate serine residues of IRS-1 and thereby diminish insulin signal transduction have been identified. Potential triggering mechanisms such as mitochondrial dysfunction, hyperinsulinemia, or hyperglycemia have also been proposed and supported by experimental and observational data. On the other hand, an additional and possibly complementary mechanism involving increased expression of p85α has also been found to play an important role in the pathogenesis of insulin resistance under certain circumstances, such as overfeeding, gestational diabetes, and steroid- and GH-induced insulin resistance. Conceivably, a combination of both increased expression of p85α and increased serine phosphorylation of IRS-1is needed to induce clinically apparent insulin resistance. Further studies are needed in order to evaluate this hypothesis.

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Chapter 7
Techniques Used to Assess Insulin Action

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Keywords insulin, sensitivity, diabetes, obesity, glucose metabolism, glucose clamp, intravenous glucose tolerance test, oral glucose tolerance test

Introduction

Despite periods of fasting and feeding, the blood glucose concentration is maintained within a relatively tight range in healthy, normal individuals. This exquisite control of the blood glucose level is achieved by the balance between glucose production and glucose uptake in the fasting state, and glucose absorption, production, and uptake in the fed state. Insulin plays a central role in the regulation of the blood glucose concentration owing to its ability to suppress glucose production and stimulate glucose uptake. In addition, insulin stimulates cell growth and promotes storage of nutrients via stimulation of lipogenesis, glycogen and protein synthesis, and inhibition of lipolysis and protein degradation. Therefore, a deficiency in the amount and/or action of insulin results in profound changes in glucose, lipid, and protein metabolism.

A deficiency in insulin action, commonly termed *insulin resistance*, is a characteristic feature of several metabolic diseases including obesity and type 2 diabetes. To understand the etiology of insulin resistance and the efficacy of treatments to prevent or reverse insulin resistance requires a measurement based upon a quantitative relationship between plasma insulin concentration and some measurable insulin-dependent process (1, 2). Most methods used to evaluate insulin action define insulin sensitivity/resistance on the basis of the ability of insulin to regulate the blood or plasma glucose level and glucose metabolism. Therefore, this chapter will describe and evaluate current methods used to assess insulin action on glucose metabolism *in vivo*. These techniques, for the most part, do not allow an evaluation of insulin action on individual tissues, but give a more integrated view of what is occurring at the whole-body level, which ultimately dictates the level of glycemia achieved. It should be noted that several excellent reviews on mechanisms and measurement of insulin action are available (1–7).

P.S. Zeitler, K.J. Nadeau (eds.) *Insulin Resistance: Childhood Precursors and Adult Disease*,

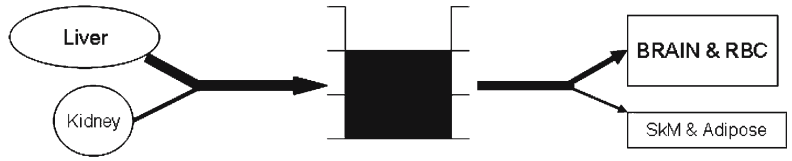
Euglycemic, Hyperinsulinemic, or Glucose Clamp

In the post-absorptive (fasting) state, the rate of glucose production from the liver and kidney is essentially equal to the rate of glucose utilization, resulting in a relatively constant level of glucose in the blood (Fig. 7.1A). Administration of insulin suppresses glucose production and stimulates glucose utilization, and therefore a reduction in the blood glucose level would be anticipated in this scenario (Fig. 7.1B). The glucose clamp takes advantage of these actions of insulin and counters the fall in glucose by using an exogenous glucose infusion calculated to match the sum of the reduction in glucose production and stimulation of glucose utilization caused by insulin administration (Fig. 7.1C). The rate of glucose infusion required to maintain post-absorptive glucose concentrations becomes an estimate of the net effect of insulin on glucose production and utilization. Therefore, insulin-sensitive subjects will be characterized by higher rates of glucose infusion relative to insulin-resistant subjects. Independent estimation of glucose production using tracers (8–11) allows the investigator to quantify the independent effects of insulin on glucose production and glucose utilization (1, 12).

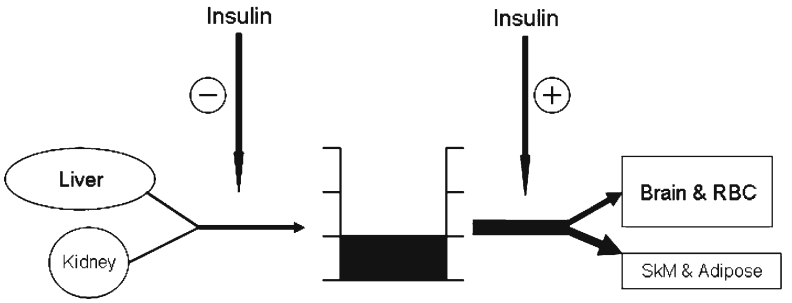
The ideal clamp protocol involves a pre-clamp period during which glucose concentration and kinetics (production and utilization) are assessed in the post-absorptive state. This period is immediately followed by a glucose clamp period in which insulin and glucose are infused and rapid blood samples taken for the measurement of glucose concentration and glucose kinetics. Such a protocol allows the investigator to match the glucose concentration during the glucose clamp period to each subject's post-absorptive glucose concentration. Estimation of insulin action can then be made on the basis of the relationship between the change in the insulin concentration (insulin during glucose clamp – insulin during post-absorptive period) and (a) the change in glucose production and (b) the change in glucose utilization. Such an approach requires at least a 4–5-h experimental period (2 h for pre-clamp and 2–3 h for clamp) but obviates many issues inherent in the glucose clamp technique, such as the difficulty in matching insulin levels in humans (1, 13, 14). When tracers are not used, pre-clamp blood samples are still required and the target glucose concentration during the clamp should be similar across all subjects. Although this approach results in clamping glucose concentration in some subjects above or below their pre-clamp values, it allows assessment of the net effects of insulin on glucose production and utilization based on the glucose infusion rate without correction for differences in glycemia (1, 15).

a. Important Issues.

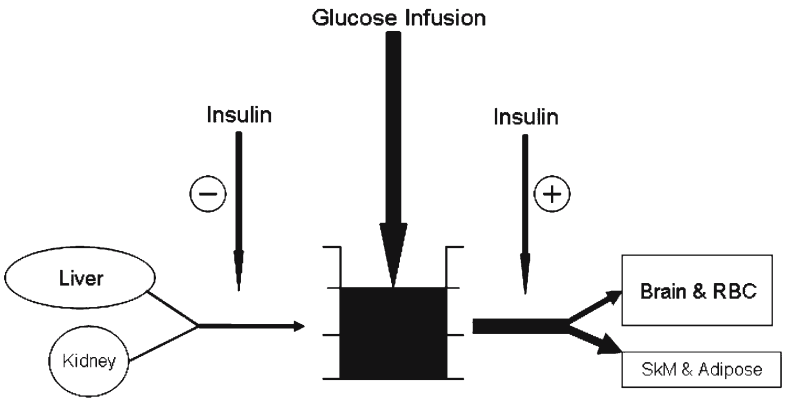
1. Sampling site. Arterial or arterialized-venous blood should be employed, as the intent of the clamp is to match the glucose concentration perfusing glucose-utilizing tissues (1, 12, 16). As insulin-stimulated glucose uptake will differ between subjects with different degrees of insulin resistance, and/or in response to an intervention, clamping venous glucose levels will result in different arterial glucose levels.



A GLUCOSE PRODUCTION BLOOD GLUCOSE GLUCOSE UPTAKE



B GLUCOSE PRODUCTION BLOOD GLUCOSE GLUCOSE UPTAKE



C GLUCOSE PRODUCTION BLOOD GLUCOSE GLUCOSE UPTAKE

Fig. 7.1 Glucose production and utilization: **(A)** In the post-absorptive state, glucose concentrations are maintained by a balance between glucose production by the liver and kidney and glucose utilization; **(B)** Insulin suppresses glucose production and stimulates glucose utilization in muscle and dipose tissue, which, if not attended to, will result in a fall of glucose levels; **(C)** Initiation of a glucose infusion in the presence of insulin serves to maintain glucose levels at post-absorptive values

2. Sampling frequency. Sampling frequencies of 5–10 min should be employed. Critical to the choice of sampling frequency is the duration of time required for equilibration of the glucose concentration following a change in the glucose infusion rate (~3–4 min) (1, 12, 16).
3. Insulin infusion and plasma insulin concentration. To bring insulin into equilibrium with its body distribution compartments most rapidly, a priming protocol should be employed, typically one in which insulin is infused at exponentially declining rates over a 10-min period (1, 17). As alluded to above, it is not possible to match insulin levels in diverse groups at the same insulin infusion rate because of variations in insulin clearance and the ability of insulin infusions to suppress endogenous insulin secretion. Therefore, it is important to incorporate plasma insulin concentrations in the analysis of insulin action during glucose clamps.
4. Steady state. Although the plasma insulin concentration following initiation of an insulin infusion will attain an apparent steady state within 30 min, the maximal effect of any given insulin concentration on glucose production and utilization requires a much longer period to stabilize. Objective criteria for the attainment of steady state, in terms of glucose metabolism, such as a time interval when the calculated rate of change in the glucose infusion rate is not significantly different from zero or is constant, should be established a priori (1, 14, 18).
5. Tracer-determined glucose appearance and disappearance. Prior to employing tracers to estimate glucose production and utilization, careful consideration must be given to the insulin infusion rate chosen, the population being tested, and the experience of the investigating team (1, 8, 10, 19, 20). In the latter case, specific expertise with both sample analysis and interpretation will ultimately prevent large potential errors. It should also be noted that in animal models, tracer approaches can be used to estimate individual tissue glucose uptake and therefore individual tissue insulin action *in vivo* (11, 21).

Thus, with the glucose clamp technique the complexity rests with the procedure itself. Continuous infusion of insulin and glucose is required with continual glucose measurements and adjustment of the glucose infusion rate to maintain predetermined glucose levels. Calculation of insulin sensitivity, however, is relatively straightforward.

Intravenous Glucose Tolerance Test

The glucose clamp technique has been suggested to be the “gold standard” approach for assessment of insulin action, primarily owing to the unambiguous end points used to determine insulin action on glucose metabolism (2, 4). However, the physiologic environment present during the glucose clamp is far removed from conditions *in vivo* during which insulin action is most critical, such

as the postprandial setting. In the postprandial setting, glucose and insulin levels undergo dynamic changes and, therefore, it is important to also be able to evaluate insulin action in this context.

There are two primary procedures used to evaluate insulin action under conditions in which glucose and insulin levels are allowed to change dynamically. In the Frequently Sampled Intravenous Glucose Tolerance Test (FSIGTT), a bolus injection of glucose (~0.3 g/kg body weight) is administered intravenously over a 30–60-s interval. Blood samples are then collected over a 3–4-h period. In the modified FSIGTT, used in subjects with insufficient insulin response, insulin is provided intravenously (0.03–0.05 U/kg body weight) 20 min following glucose administration. In both of these tests, 12–30 blood samples are required, and glucose, insulin, and sometimes C-peptide are analyzed (1, 4, 22–25).

The most widely used and quantitative approach to the analysis of insulin and glucose data derived from an FSIGTT involves minimal model assessment (1, 2). The minimal model provides two end-point parameters: the insulin sensitivity index or S_i and glucose effectiveness or S_G. S_i reflects the ability of insulin to enhance glucose uptake and inhibit glucose production and has a close numerical relationship with insulin sensitivity measured by the glucose clamp (26, 27). S_G, theoretically, is a reflection of the ability of glucose itself to stimulate glucose uptake and inhibit glucose production, but is not an insulin-independent parameter and therefore must be interpreted with caution (4, 28, 29). Toffolo and Cobelli have developed a more reliable insulin-independent glucose disposal portrait using a “hot” IVGTT two-compartment minimal model (30). This approach employs labeled (radioactive or stable isotope) glucose and a new two-compartment model to more reliably estimate insulin-independent glucose disposal. In addition, Bayesian techniques, which enable prior knowledge to be incorporated into parameter estimation routines, offer advantages in the measurement of S_i with the minimal model (23, 31–33). In particular, Bayesian approaches may allow the minimal model, which was originally specified for a single individual, to be extended to population analysis (34).

In insulin-sensitive subjects with normal beta-cell function, plasma glucose frequently decreases below fasting concentrations during the insulin-modified FSIGTT (35, 36). This decrease can result in a counter-regulatory response and underestimation of Si (36). To overcome this possibility, it has been suggested that in insulin-sensitive subjects the administered insulin dose should be as small as possible, and the plasma glucose profile carefully checked for values in the hypoglycemic range (36). Another issue related to the FSIGTT involves how best to assign basal glucose concentrations in minimal model analysis. For example, in patients with chronic heart failure, greater precision and discriminatory power of Si estimates were obtained when the basal glucose concentration was taken as the plasma glucose concentration 180 min after the start of the test (37).

In contrast to the glucose clamp, the FSIGTT procedure is relatively straightforward, and the complexity involves the computational techniques necessary to calculate S_i and S_G. While both the glucose clamp and FSIGTT are logical and viable choices for the laboratory assessment of insulin sensitivity, neither is amenable to the clinical setting or to large-scale population studies.

Measurements Involving Fasting Insulin and Glucose

Increased fasting insulin concentration in the presence of normal or elevated fasting glucose concentrations is a hallmark feature of obesity, pre-diabetes, and type 2 diabetes (Fig. 7.2). In fact, insulin resistance in terms of glucose metabolism can be defined as a requirement for more insulin to maintain normal glucose homeostasis. Two models, the Homeostatic Model Assessment (HOMA) (38) and the Quantitative Insulin Sensitivity Check Index (QUICKI) (39), take advantage of the relationship between fasting insulin and glucose for the analysis of insulin sensitivity. The appeal of this approach is evident; all that is needed is one or more consecutive fasting blood samples and subsequent analysis of glucose and insulin concentrations. Critical to the accuracy of these methods is the accurate and standardized measurement of insulin and glucose.

Homa

HOMA is a computer-based model in which a spectrum of fasting plasma insulin and glucose concentrations representative of varying degrees of β-cell deficiency and insulin resistance are plotted. The resultant array estimates insulin resistance

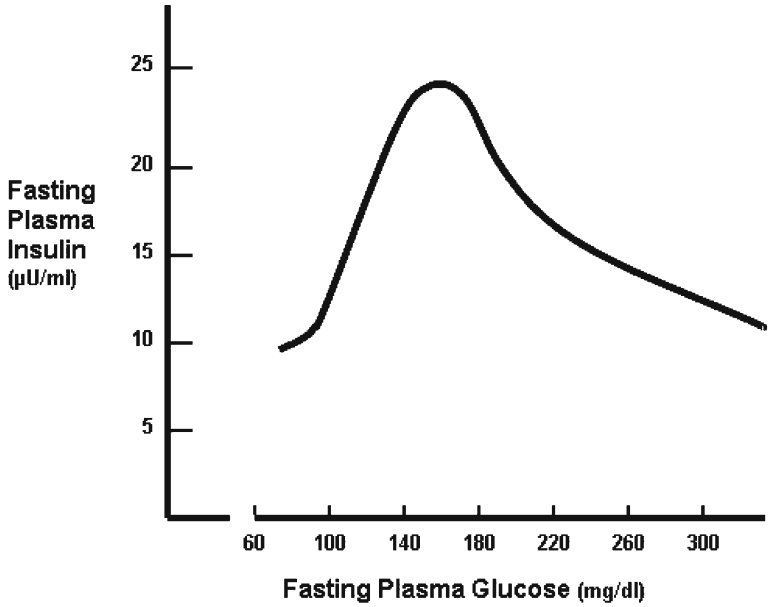


Fig. 7.2 General schematic of the relationship between fasting plasma glucose and insulin concentrations. As the fasting glucose concentration increases to a value of ~140 mg/dl, there is a progressive increase in fasting insulin concentrations. Thereafter, further increases in fasting plasma glucose are accompanied by a decline in fasting insulin concentrations (90)

and β-cell function on the basis of fasting glucose and insulin levels observed in a given subject (38). In contrast to the original HOMA model, in which nonlinear solutions were approximated, the updated HOMA model has nonlinear solutions, and therefore the latter should be used when HOMA is compared with other models (40). The updated model has also been recalibrated so that it is in line with current insulin assays, and extended to allow for the use of C-peptide if required (40).

HOMA has been used with relative success in a large number of studies and is particularly suited to large cohort studies, cross-sectional epidemiology studies, and physiologic studies in the normal population (40–42). Although cross-cultural studies using HOMA can be performed, establishment of a "normal" HOMA insulin action value is required in each comparative group studied. For example, a recent study suggested that HOMA index could be useful as a method to detect children and adolescents with metabolic syndrome but that specific HOMA cut-off values needed to be defined in the pediatric population (43). Importantly, the HOMA model has not been validated in rodents or other animals. Finally, HOMA data is typically not normally distributed and therefore should be logarithmically transformed and reported as geometric means with appropriate measures of dispersion (40).

Quicki

The QUICKI was first described in 2000 (39) and is a nonlinear transformation of HOMA rather than a novel index (4). In the initial study, QUICKI was compared to both the glucose clamp and the FSIGTT in 28 non-obese, 13 obese, and 15 diabetic subjects. In this study, the correlation between the glucose clamp and QUICKI was reported to be good ($r = 0.78$), and a comparable correlation was obtained in an independent group of obese and non-obese subjects from a separate institution. It was concluded that QUICKI could be used as an index of insulin sensitivity obtained from a fasting blood sample and might be useful for clinical research (39).

Studies that have examined the efficacy of the HOMA and QUICKI have primarily relied on the correlation coefficient achieved when compared to either the glucose clamp or FSIGTT. In such a comparison, it is important to consider whether the indices are structurally related or are correlated but based on different theoretical principles (4, 40). Results from dynamic tests are likely to be systematically different from a steady-state test like the glucose clamp, and each of these is likely different from tests that are based on basal measurements. Thus, whereas a correlation coefficient provides information on the relationship between two tests, it does not necessarily provide information pertaining to the validity or superiority of one test over another.

With this in mind, there is reasonably to good correlation ($r = 0.58$–0.88) between estimates of insulin sensitivity derived from HOMA and the glucose clamp (glucose infusion rate) and between HOMA and the minimal model (S_i) (44–48).

A number of studies have evaluated the QUICKI index (along with HOMA) in relation to the glucose clamp and/or the FSIGTT in various populations, including morbid obesity and type 2 diabetes (49, 50). These studies have suggested that indices based on fasting measurements of glucose and insulin may have clinical application, in particular, with respect to follow-up during treatment. In contrast, Abbasi and Reaven evaluated both HOMA and QUICKI in 490 non-diabetic, healthy subjects. Although both methods were correlated with the glucose clamp, it was noted that the correlation was relatively weak ($r = 0.60$). The authors suggested that these comparisons did not provide support for the superiority of QUICKI over other commonly used surrogate measures of insulin action, such as HOMA or even fasting insulin concentration.

Regular aerobic exercise is typically associated with improvements in insulin sensitivity (51). Although controversial, it has been suggested that QUICKI does not accurately reflect changes in insulin sensitivity with exercise on the basis of the fact that (a) changes in minimal model-derived S_i and QUICKI in response to exercise were not correlated, (b) QUICKI was not a robust measure of insulin sensitivity across a wide spectrum of insulin sensitivity values, and (c) discordance existed between changes in minimal model-derived S_i and changes in fasting insulin and glucose levels following exercise training (52).

In conclusion, insulin sensitivity indices based on fasting glucose and insulin are easily obtained and, for the most part, correlate at a statistically significant level with other more established methods, such as the glucose clamp and FSIGTT. Whether the discrepancies noted between these fasting indices and the more established glucose clamp and FSIGTT techniques reflect differences in accuracy, validity, or simply differences in physiology that determine the output variable is presently unclear. Also unclear is whether the HOMA and QUICKI models provide significantly more information than a fasting insulin level. Finally, it is important to appreciate that not only does fasting insulin bear a nonlinear relationship to directly measured insulin action but it likely also fails as a surrogate measure of insulin action when there is even a subtle β-cell failure (53).

Insulin Tolerance Test (ITT)

The ITT originated as a diagnostic tool for growth hormone deficiency (54). For analysis of insulin action, the standard protocol involves an insulin dose of 0.05–0.1 U insulin/kg body weight, blood sampling prior to and at 3-min intervals over a 15-min period following insulin injection (short protocol) or at 3-min intervals over 15 min followed by blood samples at 20 and 30 min (long protocol). Glucose is often provided at 15 or 30 min to prevent hypoglycemia. The rate constant for plasma glucose disappearance (K_{ITT}) is calculated from the slope of the least-squares analysis of the plasma glucose concentrations 3–15 min after intravenous insulin injection. However, newer methods have been proposed for analysis of the decline in plasma glucose (55). Implicit to this method is that the calculation of K_{ITT}

occurs over a period characterized by a linear decline in glucose concentration and prior to a glucose level that elicits a counter-regulatory response (56–60).

In 1991, the Department of Health in the United Kingdom issued a warning of the potential hazards of the ITT, stating "the insulin tolerance test should not be used in children when only growth hormone reserve needs to be tested" (61). However, it would appear, on the basis of careful evaluation of ITTs performed over a 10-year period, that the ITT is relatively safe in children provided a strict ITT protocol is followed (61). In a study performed in obese subjects, low (0.05 U/kg) and usual (0.1 U/kg) doses of insulin produced similar values of K_{ITT} (57). Studies that have examined the reproducibility of the short ITT have produced equivocal results. For example, in one study of 18 healthy subjects, the mean within-subject coefficient of variation (CV) was 13% and between-subject CV was 26% (62). In contrast, a study performed in 16 health volunteers produced a mean within-subject CV of 30.7% (63). A key difference between these two studies was the use of arterialized venous blood in the former and venous blood in the latter. It is likely that the use of arterial or arterialized venous blood is the preferred sampling method with this technique. In a study that compared the ITT with HOMA in subjects with type 2 diabetes, there was a significant but low correlation between the two tests, suggesting that the two measures should not be considered interchangeable estimates of insulin sensitivity (64). However, both the indices were significantly related with the components of the insulin resistance syndrome, and therefore it may be that these indices represent different metabolic aspects of insulin resistance and related metabolic abnormalities.

Oral Glucose Tolerance Test and Meal Glucose Tolerance Test

The oral glucose tolerance test (OGTT) has a long history as a tool to examine glucose tolerance, insulin sensitivity, and β-cell function (65–67). The limitations involved in using the traditional OGTT as an estimate of insulin sensitivity have been extensively discussed by others (1, 4, 68). The basic procedure for the OGTT involves ingestion of 75 g of glucose in ~300 ml of water over a defined period of time, typically 5 min or less (in children, 1.75 g/kg to a maximum dose of 75 g). Although this is the standard procedure, in some cases it may be valuable to use a glucose load based on body size, particularly when making comparisons between groups that vary in body weight or weight change following an intervention. Blood samples are drawn prior to and at 30-min intervals following ingestion over a 3-h period (67, 69). The more recent use of the meal glucose tolerance test (MGTT) involves ingestion of a mainly solid meal, containing 75 g carbohydrate, within ~10 min. Blood samples are collected twice prior to meal ingestion, every 10 min for the first hour following meal ingestion and at 75, 90, 120, 150, 180, 210, and 240 min (70).

OGTT and MGTT are relatively simple tests that activate the insulin–glucose homeostatic feedback process and, in principle, provide information on insulin

sensitivity in the setting where it is critically important. Interpretation is complicated, however, given that the role of the liver becomes significantly more important, both with respect to glucose uptake and production (71). In diabetic subjects, the insulin response may be insufficient for activation of glucose uptake to an extent that allows accurate assessment of insulin sensitivity. This may be one reason for the lower correlation between the OGTT and glucose clamp in diabetic subjects (72, 73).

More recent approaches have attempted to overcome some of these complications. Matsuda and DeFronzo (73) developed an index of whole-body insulin sensitivity that considers the relationship between the fasting insulin and glucose levels with those achieved during the OGTT. Soonthornpun et al. (69) developed an equation for analysis of insulin and glucose concentrations obtained from an OGTT that attempts to consider the contribution of glucose production. Comparison of insulin sensitivity based on this new equation with the glucose clamp using 33 healthy volunteers resulted in a Pearson correlation coefficient of 0.87. Caumo et al. (70) introduced an approach that allows estimation of insulin sensitivity from orally ingested glucose from an OGTT or MGTT in normal subjects. The method is based on the minimal model of glucose kinetics with the addition of an equation that describes the rate of appearance of glucose into the circulation after ingestion (1, 70). This method provides an estimate of S_i in an individual based on simple area-under-the-curve-based calculations. In normal subjects, this method provided an estimate of S_i that was in close agreement with S_i calculated from a FSIGTT. In an effort to further develop protocols such as these, a recent study performed a 5-h, 11-sample OGTT in 100 individuals and a 7-h, 21-sample MGTT in another group of 100 individuals (74). The results from this study suggested that a 7-sample, 2-h MGTT or OGTT can provide accurate assessment of both insulin action and secretion in non-diabetic humans using a minimal model-based approach. Aloulou et al. (75) have suggested that the MGTT can provide accurate evaluation of Si, as well as glucose effectiveness, using either a minimal-based approach or by simple empiric formulas in subjects with a wide range of glucose tolerance.

In summary, the OGTT or MGTT appears to provide a reasonable assessment of global glucoregulation, which includes both a physiologic description of the response to a carbohydrate load and a relatively precise analysis of insulin sensitivity and glucose effectiveness (75). The ability of this methodology to effectively evaluate insulin sensitivity and glucose effectiveness in a wider range of populations and disease states, however, requires more research.

Special Considerations

Standard measures of insulin sensitivity obtained from the glucose clamp, FSIGTT, fasting measures, or OGTT/MGTT do not account for the dynamics of insulin action: that is, how fast or slow insulin action reaches its maximal effect. Pillonetto

et al. have recently defined a new insulin sensitivity index that incorporates information on the dynamics of insulin action (76). The relationship between this new index (76) and the classic S_i parameter (1) can be demonstrated mathematically via multiplication of S_i by an efficiency term that varies from zero to 1. Since S_i (1) estimates the maximal response of insulin to control glucose, the new index reflects the fraction of the maximal response that becomes promptly available. When insulin action is rapid, efficiency will approach 1 and the new index approaches S_i; when insulin action is slow, the new index will be reduced relative to S_i. Thus, the strength of the new index is that it describes the conversion of an individual's potential insulin action resource into effective performance.

There is renewed interest in measurements of insulin sensitivity during puberty, and in children with increased risk for impairments in glucoregulation. Studies that have employed the FSIGTT or glucose clamp have documented that puberty is associated with a decrease in S_i in combination with multiple other physical and hormonal changes (77–79). Logically, surrogate measures similar to those described above are required for the clinical assessment of S_i in this population (80–88). Studies that have examined the efficacy of surrogate measures of S_i in children have produced equivocal findings and conclusions. A large reason for such discrepancies may be related to the parameters used to evaluate the efficacy of these methods. Thus, several studies demonstrate significant correlations between fasting-based methods and the FSIGTT or clamp, whereas other studies demonstrate that the specificity and sensitivity of surrogate indices are unsatisfactory. In particular, one study demonstrated that neither HOMA nor QUICKI was able to detect a reduction in insulin sensitivity with obesity or during growth hormone therapy, in contrast to the minimal model (86). It would appear that caution is warranted when using current surrogate measures of insulin sensitivity in this population.

Conclusions

The choice of methods for the assessment of insulin sensitivity in epidemiologic studies and in routine clinical practice is very different from that in the laboratory. In the former, decisions must be based on feasibility, cost, and accuracy. In this regard, reference methods such as the glucose clamp, FSIGTT and even OGTT may not be applicable, especially in the context of large population studies. In many cases it appears that fasting-based measurements can be considered not only feasible but also relatively precise, at least with respect to the relationship between these surrogate indices and clamp- or FSIGTT-based results. Clearly, caution is warranted in the interpretation of any method used to evaluate insulin sensitivity, and in particular, methods that have not been adequately studied in populations at increased risk for insulin resistance, diabetes, and the metabolic syndrome. Currently there is a lack of reference values for surrogate measures of insulin sensitivity in terms of defining cut-offs for what would be considered "impaired" vs.

"normal" insulin action, and therefore quantitative comparison between studies remains difficult. Although at least one recent study has attempted to define cut-off points for insulin resistance (89), additional studies are required to determine whether this is feasible.

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Chapter 8
Adiposity is The Enemy: Body Composition and Insulin Sensitivity

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Keywords adiposity, visceral fat, fitness, puberty, insulin sensitivity

Introduction

For decades, the link between increased body fat and insulin resistance has been recognized. So, why a whole chapter on a simple, well-established concept? Perhaps because this topic is not as crystal clear as it appears at first glance. It was once thought that declining insulin sensitivity was associated with age – the older you get, the more insulin resistant you become. However, since the advent of advanced imaging techniques in the 1980s and 90s, it has become apparent that insulin resistance is actually associated with the increase in body fatness that occurs during aging, rather than aging *per se*. In addition, these imaging techniques have fuelled the debate about which regional adipose depot – total body fat, subcutaneous fat, visceral fat, or deep subcutaneous fat – contributes most significantly to insulin resistance. This debate, in turn, has spurred investigation into the mechanism(s) behind the detrimental effect of body fat on insulin sensitivity. Finally, we are faced with the "chicken and egg" scenario: which comes first, increased adiposity or deteriorating insulin sensitivity? Superimposed on these issues is the confounding effect of the many different methods used to assess insulin sensitivity and body composition by different investigators. So, the goal of this chapter will be to unravel some of the literature dedicated to assessing the effect of body composition on insulin sensitivity and to track the effect of body fat on insulin sensitivity through the human lifespan – from childhood precursors to adult disease. The focus will be on the most recent literature, with the data on childhood and adolescence taking prominence, as other texts have comprehensively reviewed the adult literature in the past.

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Assessment of Body Composition

The most common methods currently used to assess body composition are waist circumference, waist–hip ratio, abdominal height, skin fold thickness, body mass index (BMI), bioelectrical impedance (BIA), dual emission X-ray absorptiometry (DEXA), computed tomography (CT), and magnetic resonance imaging (MRI). Each of these techniques has a dedicated purpose and its own usefulness in different settings. Underwater weighing (UWW) was originally the "gold standard" method for calculating body composition by estimating a person's total density in water. Body density can be calculated by dividing body mass by volume, with volume determined by the amount of water displaced by an individual or by the difference between the weight in water and weight in air, after making corrections for the volume of air in the lungs and the gut. Once the density is determined, equations can be used to estimate the corresponding degree of body fat, and by subtraction, fat-free mass. However, this technique requires a large sealed tank of water that must be maintained and is extremely stressful for the subjects who must repeatedly hold their breath underwater for long periods of time. Also, UWW is too strenuous to be utilized in special populations, such as children or anyone with a physical impairment or breathing abnormality. With the validation of DEXA, however, UWW is rarely used nowadays.

DEXA is recognized as the current gold standard for estimating total body fat, lean body mass, and bone mass. This technique was originally time consuming (about 40 min per scan, during which time the subject must lie very still) and relatively expensive but has become faster and cheaper in recent years. Current generation DEXA apparatus take less than 5 min to conduct a whole-body scan. Thus, this technique is now cheaper and more suitable for subjects who have difficulty lying still for extended periods of time, particularly children. DEXA technology does use ionizing radiation to capture data and, although the radiation exposure is regarded as minimal (much less than that for chest X-ray), this is a consideration when using this method in populations who already receive a high radiation dose, such as subjects on a radiotherapy regime.

BIA is an alternative method for estimating total body fat and fat-free mass, which uses the electrical resistance and reactance between two sets of electrodes to calculate body composition. However, BIA directly measures differences in body water, which are extrapolated to reflect differences in body composition. Therefore, this tool cannot be used in situations that can significantly alter total body water, such as weight loss, or to assess differences in body composition before and after an exercise regime. This severely limits the usefulness of this tool in a research setting. Also, placement of the electrodes can affect the reliability of BIA, with the greatest accuracy being achieved by using hand–foot electrode placement versus foot–foot placement. Additionally, the specific prediction equation utilized can significantly impact the accuracy of this tool. A final limitation to BIA is due to differences in cross-sectional area. The BIA current is most altered by parts of the body that have the highest resistance (e.g., the limbs); however, these segments do

not contribute nearly as much to the overall fat-free mass as other body parts, such as muscles. Therefore, smaller differences in conductance through the limbs can have a major (albeit not accurate) impact on BIA measures of body composition. In fact, multisite skin fold thickness measurements, which require no specialized electrical apparatus, have been shown to be more accurate (vs. DEXA estimations) than BIA.

With 70–90% of total adipose tissue located subcutaneously, skin fold thickness can be used to predict total body fat. The skin fold values can be compared to grouped values or norms, or can be incorporated into specific equations that can predict body density, total body fat mass, or percent body fat. These equations can predict body fat with errors between 3.5 and 5%. Once again, skin fold thickness has its limitations – single-site measurements are not as accurate or reproducible as multisite measurements and technicians must be carefully trained to avoid inter-person variation in skin fold measurements. Other sources of error with this technique include the type of caliper used and difficulty with obese patients who are too large for the caliper.

However, skin fold thickness remains a common method of estimating body composition, particularly in large cross-sectional studies or studies where the risk of radiation exposure is deemed inappropriate.

Some groups have tried to use region-of-interest analysis of DEXA scans to estimate visceral adiposity, but CT or MRI remains the method of choice for estimating this parameter. Even among those investigators using CT or MRI to assess visceral adiposity, there is debate as to the most suitable method. CT and MRI imaging are expensive, and investigators are usually charged per slice (i.e. one, single cross-sectional image). So, many investigators estimate visceral fat using single-slice CT or MRI imaging at the umbilicus or between specific vertebrae (usually L4–L5). Others, however, estimate visceral adiposity using multiple slices at various specific sites and calculate the average visceral fat area. While this method is the most accurate, it is also the most expensive and time consuming. Therefore, it is common to see single-slice CT and MRI measurements of visceral adiposity in the literature.

Waist circumference, waist–hip ratio, and abdominal height are all used as surrogate measures of total trunk fat or visceral adiposity. All seem to correlate reasonably well with the CT estimation of visceral adiposity and with markers of cardiovascular disease and the metabolic syndrome. So, while crude, the waist circumference, waist–hip ratio, or abdominal height method is commonly employed in large cross-sectional or multisite studies where CT or MRI scans are cost-prohibitive. However, CT or MRI is still the method of choice for prospective studies looking for small changes in visceral adiposity.

This is a very protracted discussion of the estimation of body composition, meant to indicate the complexity of assessing this variable and, therefore, the difficulty associated with interpreting the relevant literature. For a more comprehensive description of this topic, see a recent review by Heymsfield et al. (1). In short, the most accurate studies that measure body composition will do so in a relatively large cohort, especially when looking for small changes, utilizing DEXA

and CT or MRI scans to estimate body fat and distribution. However, it is common to see surrogate, but valid, methods used to estimate body fat, such as skin fold thickness, waist circumference, or abdominal height, in the literature, particularly for population studies or large, multicenter protocols. There is some variation in the data between different studies, which may be, at least in part, due to the sensitivity of the method used to calculate adiposity. Despite these differences, overall the literature paints a picture of adiposity as the enemy: that is, the more body fat you have, the more insulin-resistant you are.

Starting at the Very Beginning: Postnatal Influences on Body Composition and Insulin Sensitivity

There is considerable evidence that prenatal factors, such as maternal diet and alcohol intake, can influence body composition and insulin sensitivity in later life. These factors are the subject of a separate chapter in this text. So, we will begin our discussion at the beginning of the lifespan with postnatal effectors of adiposity and insulin resistance. The factors from infancy that most strongly correlate with insulin resistance and metabolic syndrome are low birth weight (either small for gestational age or premature birth) and rapid, early "catch-up" growth in early childhood (≤3 years).

Low birth weight

Low-birth-weight infants fall into two categories: (a) those considered to have complete gestation periods but are small for gestational age (SGA); and (b) those who are significantly premature at birth, with gestational periods of less than 37 weeks. Both groups are significantly insulin-resistant compared to their average birth-weight peers (2, 3). This disparity in insulin sensitivity occurs very early on – it has been demonstrated in SGA children at only 1 year of age (4) and persists through the pre-pubertal period (10–15 years) (3, 5). When obese Japanese children (mean age 10 years) are grouped into quartiles on the basis of birth weight, both low birth weight and current visceral fat mass are independently correlated with insulin resistance as estimated by measures using fasting glucose and insulin (3). In fact, visceral fat accumulation was the factor that most strongly correlated with insulin resistance, implying that it is fat gain, specifically visceral fat gain, that amplifies insulin resistance in this population, with low birth weight contributing less to this phenomenon.

The effects of low birth weight on adiposity and insulin sensitivity persist beyond childhood and puberty. It has been shown that healthy young males who were born SGA have higher body fat, in particular higher visceral fat mass, than age-matched controls (4). The SGA cohort also had higher insulin levels and lower

insulin secretion than their peers. Even in elderly populations, SGA individuals exhibit high body fat and low total lean body mass than age- and weight-matched control subjects (6).

Catch-up growth

Low-birth-weight infants typically exhibit catch-up growth in early childhood. That is, they gain weight quickly in order to achieve a normal growth curve. In a study of healthy young Swedish children, higher-than-average growth standard deviation scores (z-scores) between birth and 4 years of age were associated with elevated fasting insulin concentrations (2). Catch-up growth, whether in low-birth-weight infants or adults who have lost weight and then regained (either via intentional weight loss or unintentional weight loss as a result of starvation, injury, or chronic disease), is primarily associated with a gain in fat mass without an equivalent gain in fat-free mass (i.e. muscle mass). Catch-up growth, in terms of BMI, has been shown to be the result of weight accumulation, particularly fat mass with little gain in lean mass, rather than height velocity (7, 8). Several investigators have found that, compared to either weight- and age-matched controls or average gestational age children who were age- but not weight-matched, SGA children have greater total and visceral fat mass (9, 10). This increase in adiposity is accompanied by relative insulin resistance.

In particular, early catch-up growth (<3 years of age) is predictive of adult fat mass and can be correlated with central adiposity and hyperinsulinemia in young adulthood (11–14). Even infants who are lean exhibit hyperinsulinemia in response to a glucose challenge during catch-up growth later in childhood (15, 16). These data indicate that a period of rapid fat accumulation is associated with impaired insulin sensitivity. However, it is impossible from the current literature to determine whether it is insulin resistance that causes fat accumulation during catch-up growth or vice versa.

Although studies in low-birth-weight infants provide strong evidence for the correlation between low birth weight, catch-up growth, and increased adiposity and insulin resistance, these data are not without caveats. It must be remembered that, depending on the gestational age at birth, premature infants may have other postnatal medical problems that persist through infancy and beyond. For this reason, data from the SGA population may be more relevant to the general population, although this group of infants is thought to have some form of in utero and/or ex utero growth retardation. Therefore, the data from this population must also be interpreted with caution. Also, recent data suggest that, in healthy children, birth weight is unrelated to insulin resistance at 8 years of age (17, 18) or at age 50 (19). In this healthy cohort, there is no association with weight gain in the first weeks of life and body fatness or insulin resistance at 8 years of age. In fact, the best predictor of insulin resistance at 8 years of age was current body weight (17). So, these and other similar data refute the link between birth weight/catch-up growth and adiposity/insulin

sensitivity in healthy children, thereby reinforcing the notion that data from low-birth-weight infants, while useful, may not be indicative of trends in the general population.

Childhood: Growth and Adiposity

Childhood is a period of remarkable growth and development. During childhood, there is rapid growth rate for both height and weight, and insulin sensitivity remains high. In general, boys have greater insulin sensitivity than girls (2, 20), which has been demonstrated as early as age 4. This sex-based difference persists throughout the lifespan. Interestingly, in the Garemo study (2), higher insulin sensitivity in boys was associated with lower percentage of body fat as assessed by skin fold measurements.

Insulin sensitivity is associated with adiposity in childhood

Many of the studies conducted in young children focus on the spectrum of insulin sensitivity in normal, healthy children. Even early in childhood, differences in insulin sensitivity can be linked with adiposity. For example, it has been demonstrated that in very young, healthy children, between birth and 4 years of age, insulin resistance is associated with higher z-scores for height and weight (2). The same association can be found in older children, 8–13 years of age, where insulin sensitivity is negatively correlated with waist circumference (21). In recent longitudinal studies, current whole-body adiposity (measured via BMI and skin-folds or DEXA) was associated with insulin resistance (22) and with a clustering of metabolic syndrome risk factors, including elevated blood pressure, fasting glucose, and triglycerides, as well as low HDL cholesterol (23–25). In girls, this higher metabolic syndrome risk was also correlated with higher BMI and fat mass gain across the whole of childhood (i.e. at every age studied from 5–9 years of age). The rate of fat mass gain over childhood was linear for all girls, but those at greatest risk for metabolic syndrome (i.e. greater number of risk factors) showed a greater rate of fat accumulation than those with lower risk (24). Taken together, these studies show that there is a strong link between body fat and insulin resistance, even early in childhood, and that this association persists right through to the pubertal years. These data also demonstrate that this association can be observed even in the general child population without looking at children who are predisposed to increased adiposity, that is, overweight and obese children.

In studies examining overweight and obese children, a strong negative correlation between adiposity and insulin sensitivity also exists. In 9–11-year-old obese and lean boys and girls, insulin resistance was positively correlated with total body

fat and waist circumference (26). The study showed that 49% of the variance in insulin resistance could be explained by waist circumference and daily physical activity, although fitness was no longer correlated when accounting for total fat mass (26). These data point to visceral fat as a major determinant of insulin sensitivity in this population. Also, in 9-year-old lean and obese children who snore, insulin sensitivity and dyslipidemia were not associated with sleep-disordered breathing or the degree of apnea, as expected, but were positively correlated with whole-body adiposity (27). Taken together, data from lean and obese children of all ages show that adiposity is a major factor influencing insulin sensitivity, which is one of the features of the metabolic syndrome.

Effect of weight changes and physical fitness on insulin sensitivity in children

There is strong evidence from studies in lean and obese children that adiposity is the enemy of insulin sensitivity. If that is so, interventions that cause a reduction in body fat should improve insulin sensitivity. It has been established in the adult literature that weight loss via caloric restriction, physical activity, or a combination of these factors can cause weight loss and changes in body composition. Several recent studies have also demonstrated this in children. In a sample of pre-pubertal obese boys and girls, significant weight loss over 1 year was associated with an increase in insulin sensitivity as judged by homeostatic model assessment (HOMA) and quantitative insulin sensitivity check index (QUIKI) assessment (28, 29). Conversely, children in the same cohort who gained weight over the one-year period suffered significant impairment of insulin sensitivity.

In a study assessing cardiovascular fitness in a cohort of children, fitness was inversely correlated with insulin sensitivity, total body fat (DEXA), and abdominal fat (CT) (30). However, the relationship between fitness and insulin sensitivity was lost when body fatness was taken into account; so this relationship is, at least in part, mediated by body adiposity. Contrary to this data, in 8–10-year-old Danish children, physical activity (assessed using an accelerometer) was independently and negatively associated with metabolic risk factors, notably adiposity and fasting insulin, which was used as a surrogate measure of insulin sensitivity (31).

Although data demonstrating an increase in insulin sensitivity in response to weight and fat loss in young children is not abundant, it shows consistently that there is a correlation between these parameters. There is conflicting data as to whether physical activity has any effect on insulin sensitivity that is independent of changes in body fat, and further studies need to be conducted in this area to reach any meaningful conclusion. However, the fact that weight loss caused increased insulin sensitivity, whereas weight gain compromised insulin sensitivity in young children, shows that adiposity is a key factor influencing insulin sensitivity in this population.

Effect of childhood adiposity on insulin sensitivity and metabolic syndrome as an adult: childhood precursors of adult disease

Large retrospective and prospective trials show a strong link between childhood adiposity and insulin resistance and the presence of the metabolic syndrome. In a retrospective analysis of data from the United Kingdom, Martin et al. found that BMI at 2–14 years of age is negatively correlated with insulin sensitivity in lean adults at age 71 (32). In addition, childhood BMI is the strongest predictive factor of the clustering of metabolic syndrome risk factors in adulthood (33). In prospective studies, visceral adiposity, measured using waist circumference, and elevated fasting triglycerides at age 9–10 years, predict metabolic syndrome in 18–19-year-old black and white women (34). Also, intima medial thickness, a marker of negative cardiovascular health, is higher in obese than lean children (9–13 years), suggesting that cardiovascular changes occur early in childhood before any chronic disease can be detected (35). Finally, data from the Bogalusa Heart Study shows that those children with higher body fat and blood pressure exhibited decreased insulin sensitivity as adults. Furthermore, those subjects that had elevated adiposity and insulin resistance in childhood went on to become hypertensive as adults (36). Collectively, these data indicate that childhood adiposity is strongly associated with the development of insulin resistance, hypertension, and cardiovascular disease in adults. Thus, the influence of childhood adiposity on these key risk factors and on the development of metabolic syndrome *per se* indicates that the precursors of this chronic disease occur very early in life and can be accelerated by accumulation of body fat. So, adiposity is the enemy of insulin sensitivity from a very early age and can exacerbate the incidence of metabolic syndrome later in life. Thus, childhood adiposity is a key precursor to metabolic syndrome in adults. Given these data, prevention strategies for metabolic syndrome could include childhood interventions aimed at decreasing body weight and total adiposity, and increasing physical activity.

Puberty: A Period of Profound Insulin Resistance and Dramatic Body Composition Changes

Puberty is a period of profound insulin resistance that ameliorates as the adolescent transitions to adulthood (37–39). In both Caucasian and African-American cohorts, insulin resistance increased by about 30% between Tanner stages I and III (39). Hyperinsulinemic-euglycemic clamp studies conducted on healthy children during Tanner stage I and repeated at Tanner stage III–V have shown that insulin sensitivity decreases by about 50% during puberty (40). This relationship is maintained regardless of whole-body adiposity in healthy children (39, 40) and is associated with growth hormone concentrations (40). This dramatic decrease in insulin

sensitivity during puberty is offset by an increase in insulin secretion, facilitating glucose homeostasis (40).

Insulin sensitivity is associated with adiposity in adolescents

In girls, age at menarche, rather than chronological age, is correlated with insulin sensitivity and cardiovascular disease risk (41). Specifically, young menarcheal age is associated with insulin resistance and increased cardiovascular disease risk. This increased insulin resistance is associated with fat mass but not fat-free mass in this cohort. It has also been reported that those subjects who had an early menarcheal age were heavier, had greater total and percent body fat, and greater abdominal circumference during the course of adolescence than average- or late-maturing girls (41).

In a small sample of healthy, lean adolescents, insulin resistance at puberty was most highly correlated with fat mass (42). This data was replicated in a large population-based study in Taiwanese adolescents where total body fat, assessed via BMI, was the factor that most highly correlated with insulin resistance (43). In overweight Hispanic adolescents with a family history of type 2 diabetes, the change in insulin sensitivity across puberty was not apparent when data was adjusted for body composition, suggesting that the effect of puberty on insulin sensitivity is deleted by the extremely high adiposity and, possibly, family history in this population (44). In North American adolescents, clustering of the metabolic syndrome risk factors (three or more components) is related to body weight and total adiposity but not height velocity (45, 46). Furthermore, it seems that this relationship is most prominent among adolescents with more visceral adiposity rather than high total adiposity.

The most convincing evidence that insulin resistance is related to visceral adiposity in adolescents comes from Lee et al. and Bacha et al. (47, 48), who demonstrated that waist circumference and visceral fat assessed by CT were positively correlated with insulin resistance measured via the hyperinsulinemic-euglycemic clamp technique. This evidence, using the gold standard techniques to assess both body composition and insulin sensitivity, is corroborated by population studies which also show that insulin resistance in adolescents and young adults (14–25 years of age) is associated with high body fat, with particularly strong correlations for abdominal adiposity (42) and truncal fat as assessed by skin fold measurements at four sites (49). In addition, it has been demonstrated that ethnic differences between South Asian and white European adolescents (14–17 years of age) are associated with differences in body composition between the groups. South Asian adolescents are more insulin-resistant and have higher body fat, as estimated by DEXA, and central adiposity, measured via waist–hip ratio, than the white European group (50, 51).

Taken together, data from lean and obese adolescents show that adiposity, particularly visceral adiposity, is associated with insulin resistance. Although insulin resistance is a prominent feature of puberty, this state is enhanced with increasing adiposity, making adiposity the enemy, even during adolescence.

Effect of weight changes and physical fitness on insulin sensitivity in adolescents

Although there is some evidence that interventions to decrease weight and adiposity in obese children improve insulin sensitivity, the bulk of the data on this topic comes from lifestyle intervention studies in adolescents. A 12-week lifestyle intervention in 16-year-old obese adolescents caused a decrease in total percent body fat and an increase in fat-free mass. The reported decrease in percent body fat was associated with improved insulin sensitivity (52). Although changes in fat-free mass can confound the outcome of weight loss studies, it has been shown that a complete lifestyle intervention in obese youth with metabolic syndrome (10–17 years) caused a very modest decrease in body weight, with only slight amelioration of whole-body adiposity but was associated with an increase in insulin sensitivity despite the lack of any significant change in fat-free mass (53).

There is some question whether the improvements in insulin sensitivity that occur during lifestyle intervention programs are due to weight loss *per se* or if the physical activity component of such programs has an adiposity-independent effect on insulin sensitivity. In a cross-sectional study of post-pubertal females who were not on a weight loss regime, percent body fat was significantly correlated with insulin resistance as measured by HOMA (54). In addition, physical fitness was predictive of insulin sensitivity and, statistically, contributed more to insulin sensitivity than body fat alone. In obese, middle-school children randomized to intensive lifestyle intervention for weight loss or regular physical education classes, the lifestyle group showed weight loss and decreased total and percent body fat and increased fitness (vO_2max). Decreased adiposity and increased fitness were both associated with increased insulin sensitivity in the lifestyle group (55). Conversely, a 16-week resistance training program in overweight Latino males (average age 15 years) showed that training improved insulin sensitivity compared to control group, independent of changes in fat mass or fat-free mass (56). Utilizing a 12-week aerobic training program in obese girls (9–15 years), Nassis et al. reported increased fitness and improved insulin sensitivity, without any change in total body weight or percent body fat (57). It is important to note that in all these studies, whether or not fitness was an independent correlate of insulin sensitivity, decreased adiposity was always associated with an increase in insulin sensitivity.

In combination, these data imply that the decreased body fat associated with weight loss in obese adolescents causes an improvement in insulin sensitivity. In addition, there seems to be some further benefit of physical fitness on insulin

sensitivity, independent of body fatness, in this population which is undergoing a period of profound insulin resistance and dramatic hormonal changes.

Effect of adolescent adiposity on insulin sensitivity and metabolic syndrome as an adult : adolescent precursors of adult disease

Abundant data directly link body composition during adolescence with metabolic syndrome in adults. Studies have shown that the composite metabolic risk factors present in 15-year-old adolescents are also present in young adults at 26 years of age (58). Adolescent BMI and fasting insulin concentration have been shown to be the strongest predictors of the clustering of metabolic syndrome risk factors in adulthood (33). A study examining insulin sensitivity via the hyperinsulinemic-euglycemic clamp technique in over 12,000 adolescents showed that there is a significant correlation between total body fat and insulin resistance and that those youth who had the greatest insulin resistance also had a clustering of metabolic syndrome risk factors (59). In addition, data from the Bogalusa Heart Study shows that pre-hypertensive adults exhibit higher body fat and higher blood pressure in childhood, followed by increased fasting glucose concentrations in adolescence, and decreased insulin sensitivity as adults (36). Also, decreased cardiovascular health at 27 years of age was dependent on weight gain since age 13 (60). As all these factors are intrinsically linked with development of metabolic syndrome, adolescent adiposity is a good predictor of adult insulin sensitivity and progression to the disease state (i.e., the metabolic syndrome).

Data from both healthy and obese adolescents show that there is a strong association between adiposity and insulin resistance. Also, data from these studies in pubertal youth provide strong evidence that visceral adiposity may be a key factor in the determination of insulin resistance and future development of the metabolic syndrome.

Adulthood

The transient period of insulin resistance that occurs during puberty is normally ameliorated by adulthood. However, the transition from adolescent to adult is often associated with a decrease in physical activity and subsequent fat accumulation over many years. The rate of fat accumulation increases in old age and, specifically in females, post menopause. From the previous sections it would be tempting to assume that remaining lean during childhood and puberty would protect an adult from insulin resistance and the metabolic syndrome. However, weight gain during adulthood can also lead to detrimental changes in insulin sensitivity and development of the metabolic syndrome (Fig. 8.1).

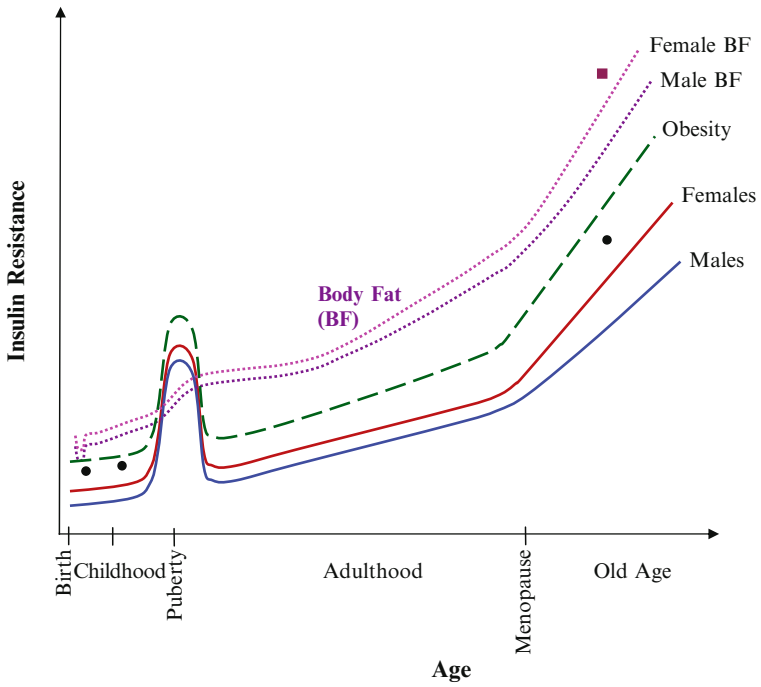

Fig. 8.1 Representation of insulin resistance and body fatness (BF) throughout life. Over all stages of the life cycle, from birth to old age, insulin resistance (solid lines) and adiposity (dotted lines) exhibit parallel trends. The only exception is puberty, a time of profound insulin resistance, which does not directly correlate with the adiposity trajectory. However, even during puberty, those adolescents with higher body fat (i.e. obese) are more insulin-resistant than their lean peers and are more likely to develop the metabolic syndrome as adults. Females are slightly more insulin-resistant and have a higher body fat percentage than males from early childhood through old age. The dots represent the insulin resistance of low-birth-weight infants at various ages, which is always higher than their normal-birth-weight counterparts. The square indicates the body fatness of low-birth-weight infants at old age. This higher percent body fat is associated with the increased insulin resistance represented by the corresponding dot (*See* Color Plate 1)

Insulin sensitivity is associated with adiposity in adults

Despite the discussion earlier in this chapter of the postnatal factors that affect insulin sensitivity, there is evidence that factors such as birth weight and early infant nutrition contribute little to insulin sensitivity at 71 years. However, adult whole-body adiposity and waist–hip ratio correlated with insulin resistance in both men and women at this age, suggest that adult weight gain and visceral adiposity are stronger determinants of the development of insulin resistance than postnatal factors (19). Many different studies demonstrate the association between current adult adiposity and decreased insulin sensitivity at various ages throughout adulthood, demonstrating the rigorous nature of this relationship (see, e.g., (61, 62). In young males

(27 years of age), left ventricular mass, a measure of cardiovascular health, was associated with insulin resistance but this relationship was dependent on the degree of adiposity [assessed via BMI (60)]. Decreased cardiovascular health at 27 years of age was dependent on weight gain since age 13 (60) (Fig. 8.2). In a study by Bryhni et al. (63), adult males were placed in three groups: (a) elderly men, 71–77 years of age; (b) young men, in their early 30s matched to the elderly men for BMI and waist circumference; and (c) young men, in their early 30s with BMI and waist circumference that were average for age. This study unequivocally showed that insulin sensitivity is related to body fat and not age, with the elderly and matched young groups having equivalent glucose disposal rates during a clamp, whereas glucose disposal in the average young males was higher [i.e., higher insulin sensitivity (63)]. In addition, higher waist circumference and fasting triglyceride concentrations were negatively correlated with insulin sensitivity. In fact, waist circumference correlated more strongly with insulin resistance than whole-body fat mass (63), suggesting that visceral adiposity is a key factor in the development of insulin resistance.

Most data in adults, that demonstrate an association between body fat and insulin resistance, also reveal that visceral adiposity is a better predictor of insulin

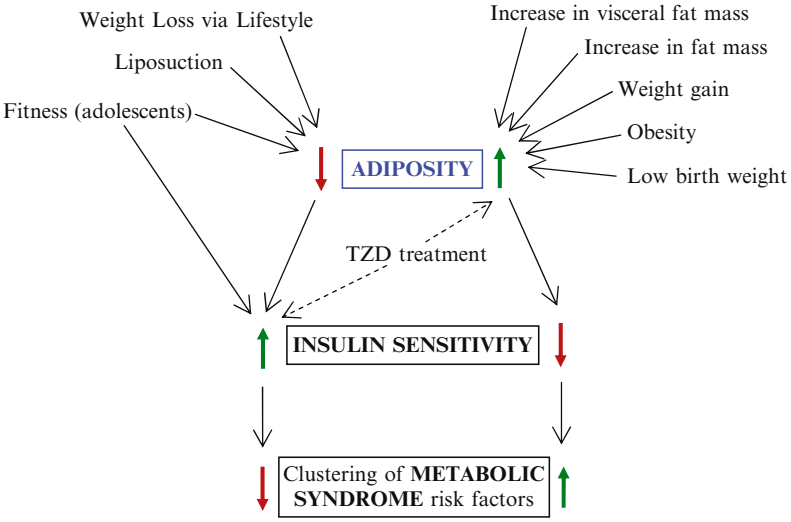

Fig. 8.2 Representation of the factors influencing whole body adiposity and, hence, insulin sensitivity. Factors that increase adiposity act to decrease insulin sensitivity and are associated with the development of metabolic syndrome in adulthood. The associations shown in this diagram apply through all stages of life – from childhood through adolescence and into adulthood. The only exception to this association between increasing adiposity and decreasing insulin sensitivity occurs in subjects treated with thiazolidinediones (TZDs), in whom whole-body fatness increases but so too does insulin sensitivity. This effect, however, is mediated through the TZD's ability to redistribute body fat to peripheral subcutaneous depots and away from visceral fat. In addition, physical fitness in adolescents may influence insulin sensitivity both via decreased total body adiposity and via a direct, fat-independent mechanism (*See* Color Plate 2)

resistance than total body fat. This relationship between visceral fat and insulin resistance exists in both men (64) and women, pre- or post menopause (61, 62, 65). In all these studies, there was a relationship between whole-body adiposity and insulin resistance, but the association between visceral adiposity and insulin resistance was stronger and explained more of the variability in insulin sensitivity among individuals. However, data also exist that show a strong relationship between visceral adiposity and insulin resistance independent of total body fat (66).

Data presented here show that body fatness, particularly visceral adiposity, is associated with insulin resistance in adults and that weight gain through the years, whether the subject was lean or obese during childhood, is detrimental to insulin sensitivity. The case for a link between visceral adiposity and insulin resistance is particularly strong in the adult literature.

Effect of weight changes and physical fitness on insulin sensitivity in adults

In overweight males and females who lost 10–14% of their initial body weight, weight loss decreased percent body fat, visceral fat, subcutaneous abdominal fat cell size (from abdominal biopsy), and intrahepatic lipid content (assessed via magnetic resonance spectroscopy). Improvement in insulin sensitivity most strongly correlated with fat cell size, although this parameter was also linked with visceral and hepatic adiposity (67). So, large fat cells seem to enhance insulin resistance, which can be ameliorated via weight loss that decreases adipocyte cell size. However, this study examined only subcutaneous abdominal adipocyte size, so it is impossible to generalize these findings to other fat depots. Therefore, it would be useful in future studies to examine and compare adipocyte cell size from other fat depots, particularly in obese subjects (e.g., gluteal, femoral fat, and, ideally, visceral adipose tissue depots). In the only study to assess the effects of weight loss on fat cell size in different body depots, insulin sensitivity improved equally whether fat cell size was reduced in the abdominal or femoral fat (68), implying that adipocyte cell size plays a role in the modulation of insulin sensitivity, possibly via the effect of adipokines.

In adult cohorts of men and women of various ages, from young adults through old age, modest weight loss, in most cases accounting for 5–11% of initial body weight, led to a significant improvement in insulin sensitivity (61, 65, 69–71). In some cases, the relationship between weight loss, fat mass, and insulin sensitivity is confounded by the fact that insulin sensitivity is also affected by changes in fat-free mass that occur during weight loss (70). However, the loss of body fat seems to be the strongest, single correlative factor and was associated with a 63% increase in insulin sensitivity. In addition, it has been shown that, when comparing caloric restriction versus physical activity weight loss interventions, there is no difference in the total amount of weight lost, fat lost, or insulin sensitivity, regardless of the method used to achieve weight loss (71). This data is in direct contrast to the data

in adolescents, which demonstrated that weight loss via physical activity might improve insulin sensitivity, independent of changes in body fat. This difference between adolescents and adults needs to be examined further and could provide valuable information regarding the optimal weight-loss diet(s) for metabolic health in these distinct populations.

In adults, as in adolescents, physical fitness has a strong influence on insulin sensitivity. In demonstration of this, women with high cardiovascular fitness, whether lean or obese, have been shown to have only marginally different insulin sensitivity indices, despite the obese women having twice as much body fat as the lean women (72). In studies in elderly men and women, both adiposity and cardiovascular fitness are related to insulin sensitivity (73). However, visceral adiposity, as assessed by waist circumference, was the strongest single factor that accounted for insulin sensitivity (73). In the O'Leary study (74), there were changes in total body weight and body fat in response to the aerobic training program without any change in fat-free mass, indicating that there is a strong correlation between visceral adiposity and insulin sensitivity in this cohort, independent of confounding body composition factors.

Although we have discussed weight loss via lifestyle interventions, it is also becoming increasingly common to lose dramatic amounts of body fat following bariatric surgery. Bariatric surgery causes dramatic weight loss, with most (more than 75%) lost weight consisting of fat (75), and can normalize insulin sensitivity and even reverse metabolic syndrome or type 2 diabetes within 1 week after surgery (76). So, in adults, as in other, younger populations, body fat predicts insulin resistance, whereas weight loss, specifically fat loss, dramatically improves insulin sensitivity. Although this association does not prove cause and effect, it provides evidence for the argument that adiposity is the enemy and reinforces the importance of a healthy lifestyle and weight maintenance through the entire lifespan to ensure metabolic health.

Body Fat Distribution: Is Visceral Fat the Culprit?

As discussed already in this chapter, modest weight loss in men and women, in most cases accounting for 5–11% of initial body weight, leads to a significant improvement in insulin sensitivity (65, 70, 71, 73). In all cases, this improvement in insulin sensitivity was associated with a decrease in total body fat but was even more strongly associated with visceral fat as judged by MRI (61), abdominal height (65), or waist circumference (73). Aerobic exercise training in older women increased fat-free mass and decreased total fat mass, comprising a large decrease in subcutaneous fat but no change in visceral fat. This training regime did not improve insulin sensitivity, suggesting that visceral fat is the key modulator of insulin sensitivity changes in response to weight loss/exercise interventions (77). In addition, the strong association between visceral fat and insulin resistance can even be detected in lean subjects in cross-sectional studies (62).

Guldiken et al. have shown that visceral fat is associated with markers of the metabolic syndrome, without any contribution from subcutaneous fat (78). In addition, insulin resistance in the adult offspring of diabetic parents is associated with high visceral fat, independent of total body fat (66). Other data show that insulin sensitivity was normalized following an exercise training program in older men and women and correlated only with changes in visceral adiposity and fitness, with visceral adiposity being the most important single factor (74).

Interesting data that address the issue of visceral vs. subcutaneous fat come from subjects on thiazolidinedione (TZD) medication. TZDs include rosiglitazone and pioglitazone, which are used as insulin sensitizers. In most studies using TZD treatment in persons with Type 2 diabetes, obese individuals, or subjects with metabolic syndrome, TZD is responsible for a significant improvement in insulin sensitivity despite significant weight gain (79). This is in part due to a dramatic increase in total body water that occurs with TZD treatment and can account for as much as 75% of weight gain in short-term trials (80). The remaining weight gain is reported to consist of abdominal and subcutaneous fat (80). In non-diabetic subjects with upper-body obesity, TZD treatment increased total body weight, primarily owing to an increase in subcutaneous fat mass, without any change in visceral fat mass. These subjects showed an improvement in insulin sensitivity despite an increase in subcutaneous adiposity (68). However, visceral fat mass did not change in this study, so either the visceral–subcutaneous fat ratio is responsible or another factor may be involved in the association of insulin sensitivity and adiposity in this case. This factor could be an effect of the treatment on fat cell size. TZD treatment improved insulin sensitivity to the same degree as weight loss via diet and exercise, although these two regimes altered fat cell size in different depots. TZD treatment decreased abdominal fat cell size more than femoral fat cell size, whereas the opposite was observed in the diet and exercise group in which femoral fat cell size was reduced more than abdominal fat cell size (68).

TZD studies show that increasing subcutaneous fat, while maintaining or decreasing visceral fat mass, and thereby changing the visceral to subcutaneous fat ratio, improves insulin sensitivity. So, what happens when subcutaneous fat is selectively removed without any change in visceral adiposity during surgical liposuction procedures? Although there are relatively few studies that have addressed this topic, all studies removed between 2.5–4.9 liters of aspirate, which corresponds to a large amount of subcutaneous fat (81–85). Weight loss during the follow-up period, which varied from 4 weeks to one year, was between 3.5 and 10.5 kg. All but one study (84) found that selective removal of subcutaneous fat results in an increase in insulin sensitivity (81–83, 85), suggesting that subcutaneous fat can affect insulin sensitivity, independent of any change in visceral mass. Small-volume liposuction research also shows that this procedure can increase insulin sensitivity (86). However, the one study that showed that liposuction does not improve insulin sensitivity (84) used only overweight and obese women, assessed insulin sensitivity via the hyperinsulinemic-euglycemic clamp technique, and was, therefore, the most rigorous in terms of scientific design. Thus, although the bulk of the data in this arena points to an increase in insulin sensitivity following subcutaneous fat removal via liposuction, more mechanistic data is necessary to resolve this issue.

The weight of evidence supports the notion that although total adiposity is related to increased insulin resistance, it is visceral fat that is the key factor involved in this association. Several studies point to visceral adiposity as the key body composition factor that influences insulin sensitivity with little or no contribution from subcutaneous adipose tissue. The major exception to this is the sudden removal of large quantities of subcutaneous fat via liposuction, which might increase insulin sensitivity, although this data needs to be carefully verified. The difference between weight loss studies using lifestyle or TZD treatment and liposuction may lie in changes in fat-free mass or fat cell size. While lifestyle and TZD treatment have been shown to reduce fat-free mass and fat cell size in different body depots, liposuction does not. Thus, more mechanistic studies are needed to resolve this issue. Alternatively, it is possible that visceral adipose tissue is a key regulator of insulin sensitivity during body weight homeostasis (i.e., when no additional subcutaneous differentiation is occurring and the existing fat cells are large) but subcutaneous fat depots may play a larger role in situations of adipocyte differentiation.

The role of deep vs. superficial subcutaneous adipose tissue has not been discussed in this chapter as there are almost no studies that have investigated this factor in children and adolescents, although deep subcutaneous and visceral fat have been associated with insulin resistance in the adult literature. So it seems that visceral fat plays a primary role in changes in insulin sensitivity, with subcutaneous fat being involved to a lesser degree. Although the data in humans to support this notion are abundant, it is not yet unequivocal.

Effect of Diet on Body Composition and Insulin Sensitivity

It has been known for many years that dietary intake can affect body composition and is a major contributor to obesity. As this is a widely documented concept and we have established the link between increasing adiposity and insulin resistance in the remainder of this chapter, the effect of diet on body composition and insulin sensitivity will be addressed only briefly here [for a comprehensive review see McCauley *et al*. (87)]. In particular, the studies presented in this section represent those studies that examine the effect of the diet acutely on insulin sensitivity, thereby determining whether diet can affect insulin sensitivity independent of changes in body composition. In a study of healthy white and black children, 6 to 14 years of age, a high-fat diet was associated with a decrease in insulin sensitivity, independent of body fat, only in black children (88). No association between diet and insulin sensitivity, independent of adiposity, was detected in the white cohort. However, other researchers have found that acute high-fat intake is associated with lower insulin sensitivity in both children (89) and adolescents (90).

Although fat intake can influence insulin sensitivity independent of changes in body composition, the effect of carbohydrate intake is, at least in part, mediated through changes in adiposity in children. High adiposity and the risk for metabolic

syndrome in 13-year-old girls are associated with the consumption of more sweetened beverages during childhood [5–9 years, no association with intake at 11 years (24)]. This difference in sweetened beverage intake accounted for an increase of 167–314 J per day. So, data in children and adolescents point to a high-fat diet that is also high in sweetened beverages as the key dietary component associated with insulin resistance in this population.

Summary: Adiposity Is the Enemy

There is a strong link between adiposity and insulin sensitivity that shows that adiposity is, indeed, the enemy (Fig. 8.1). This relationship is apparent even during the earliest years of life and persists through the whole of childhood, adolescence, adulthood, and even in old age. In addition, it has been shown that body fatness from a young age influences the clustering of metabolic syndrome risk factors in adolescence and adulthood. The strength of this relationship throughout the lifespan demonstrates that adiposity has a key influence on insulin sensitivity and development of the metabolic syndrome, from childhood precursor to adult disease. Given this data, prevention strategies for metabolic syndrome could include childhood interventions aimed at decreasing body weight and total adiposity, and increasing physical activity, especially in adolescents in whom physical activity may have an adiposity-independent effect on improvement in insulin sensitivity. These interventions should be continued throughout life, as rapid weight gain, even in old age, can impair insulin sensitivity. Conversely, the loss of body fat at any age improves insulin sensitivity.

Given that adiposity impairs insulin sensitivity and is associated with the development of the metabolic syndrome, it seems vital to achieve a healthy lifestyle and weight maintenance through the entire lifespan to ensure metabolic health.

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Chapter 9
Ectopic Fat Deposition, Adiponectin and Insulin Resistance in Obese Adolescents

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Keywords IGT, T2DM, insulin sensitivity, HOMA-IR, visceral adiposity, HFF, NAFLD, NASH, IMCL, EMCL, adiponectin

Introduction

Obesity is one of the most serious and urgent public health problems in both developed and developing countries (1). It has reached epidemic proportions in the United States. Since the 1970s, the prevalence of overweight doubled among children of 6 to 11 years of age and tripled among those 12 to 17 years of age (2). Among children aged 6 through 19 years in 2001–2002, 31.5% were at risk for overweight and 16.5% were overweight (more than 3 times the target prevalence of 5%) (3). Furthermore, the problem falls disproportionately on African-American and Hispanic children.

Many of the metabolic and cardiovascular complications associated with obesity, namely, impaired glucose tolerance (IGT), type 2 diabetes (T2DM), hypertension, dyslipidemia, and systemic "low-grade" inflammation, are already present during childhood and are closely linked to the concomitant insulin resistance/hyperinsulinemia (4) and degree of obesity (5). Moreover, these comorbidities persist into adulthood (6, 7). In the recent National Health and Nutrition Examination Survey (NHANES) III data on the prevalence of metabolic syndrome – defined by the coexistence of central obesity, dyslipidemia, hypertension, and prediabetes – approximately 4% of all adolescents and nearly 30% of overweight children and adolescents (BMI >95th percentile) had metabolic syndrome (8). One dire prediction from the centers for disease control (CDC) estimated that if current obesity rates continue, one in three babies born in 2000 would eventually develop T2DM (9).

Studies from our childhood obesity cohort demonstrated that most of the obese children do not develop alterations of glucose metabolism early in life. Therefore, the question arises whether it is not strictly the degree of obesity but the pattern of lipid partitioning in traditional depots (the visceral and subcutaneous compartments) and within insulin-responsive tissues (muscle and liver) that has the major impact on glucose metabolism in the obese child. In this chapter, we focus on the

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relations of lipid partitioning and peripheral insulin sensitivity, insulin secretion patterns, and the natural history of normal and abnormal glucose tolerance in growing, obese youth.

Prevalence and natural history of IGT in childhood obesity

In adults, the progression from normal glucose tolerance to overt T2DM involves an intermediate stage of hyperglycemia, characterized by impaired fasting glucose (IFG) and/or IGT, now known as prediabetes (10). Recent reports have documented a high prevalence of prediabetes among children and adolescents. Cruz et al. found that 28% of obese Hispanic children with a positive family history for T2DM had IGT, but found no cases of T2DM (11). Of particular interest, IGT and T2DM are far more common in obese European children of Caucasian origin than previously thought. An IGT prevalence rate of 15–20% was found in obese children in Germany. Gruters et al. (12) reported that the prevalence of IGT was 36.3% among an obese multiethnic cohort of children and adolescents with a risk factor for T2DM. Moreover, among the children and adolescents with IGT, 86% were Caucasian and 14% were non-Caucasian. High prevalence of IGT has also been reported in obese children from Thailand (13) and the Philippines (14).

Similarly, in our study of the prevalence of IGT in a multiethnic clinic-based population of 55 obese children and 112 obese adolescents, IGT was detected in 25% of the obese children and 21% of the obese adolescents, and silent T2DM was identified in 4% of the obese adolescents, irrespective of ethnicity (15). This was the first study to highlight the high prevalence of prediabetes in the midst of the epidemic of childhood obesity. The risk factors associated with IGT in our study were, in order of importance, (a) insulin resistance (estimated by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), (b) fasting proinsulin, (c) 2-hour insulin level and (d) fasting insulin. Taken together, these studies indicate that early stages of abnormal glucose metabolism may precede the full onset of clinical diabetes, as happens in adults.

Owing to the cross-sectional nature of this study, we were unable to confirm the glucose continuum in these youngsters. Therefore, a longitudinal study was needed. We followed 117 obese children and adolescents from a pediatric weight management clinic (84 with NGT and 33 with IGT) with oral glucose tolerance tests (OGTT) repeated after 18–24 months (15). Eight of the subjects with normal glucose tolerance became impaired. Eight subjects (24.2%) with IGT developed T2DM, 15 (45.5%) converted to normal glucose tolerance, and 10 remained impaired (30.3%). Transition from normal to IGT and from IGT to diabetes was associated with significant increases in weight, while conversion from IGT to NGT was associated with the least amount of weight gain. The insulinogenic index, an OGTT-based estimate of insulin secretion in subjects with IGT, specifically those who later became diabetic, was lower at baseline at 2.11 (1.18–3.78), compared to subjects with normal glucose tolerance at 3.63

(3.12–4.26, $p = 0.03$). The best predictor of the change of the 2-h glucose level was the change in insulin sensitivity.

Our data illustrated the importance of variations in weight gain on changes in glucose tolerance in childhood obesity. Most of the children grew in height and gained weight on a track consistent with their prior growth patterns, resulting in stable BMI z-scores or relative adiposity. However, the children who progressed from NGT to IGT had the largest increase in body weight and an increase in relative adiposity. Even more exciting is the observation that IGT subjects who converted back to NGT had minimal increases in body weight and a reduction in BMI z-score. Impaired glucose tolerance has been demonstrated to be reversible in adults who undertook significant lifestyle modifications resulting in a reduction in body weight. Our data suggest that, even in the absence of frank weight loss, IGT may be reversible in obese children by lifestyle interventions that are successful in maintaining a stable body weight during a period of active growth. Nevertheless, the potential for obese youth with IGT to deteriorate rapidly to T2DM makes the window of opportunity to implement such interventions very limited.

Ectopic fat deposition and insulin resistance in childhood obesity

More recently, muscle and liver fat have garnered increased attention as important contributors to the metabolic phenotype responsible for insulin resistance (16).

Lipid droplets in the myocyte

The lipid composition of skeletal muscle tissue, where most (70%) glucose disposal occurs, has recently attracted much attention as a major player in the development of muscle insulin resistance (17). Strong inverse correlation between insulin resistance and intramyocellular lipid (IMCL) content have been reported in offspring of T2DM adults, suggesting that a high IMCL content might be involved in the development of insulin resistance (18, 19). Alternatively, intramuscular lipid accumulation may be caused by a reduction of fat oxidation (20), related to low aerobic capacity or reduced sympathetic tone. In particular, the tendency to accumulate lipid within muscles may be determined by the amount and functionality of mitochondria within the myocytes and by their capacity to oxidize fat.

To gain insight into the lipid composition of skeletal muscle tissue, we used ^{1}H-NMR spectroscopy to quantify noninvasively the IMCL and extramyocellular lipid (EMCL) content of the soleus muscle (21, 22) in obese children and adolescents, either with or without prediabetes. We found an excessive accumulation of IMCL content in the soleus muscle of obese adolescents with IGT compared to age- and adiposity-matched obese adolescents with NGT. Moreover, we found that whole-body

insulin sensitivity, as assessed by the glucose clamp technique, varied as a function of IMCL stores (Pearson's correlation $r = -0.59$, $p < 0.02$) (21). These relationships were independent of percent total body fat and subcutaneous abdominal fat, but not visceral fat mass. Therefore, our data suggest that IMCL may play an important role in modulating insulin sensitivity, particularly in obese adolescents. The striking relationship between IMCL and insulin sensitivity in such a young population suggests that these findings are not a consequence of aging, but are actually expressed early in the natural course of obesity. This is the first spectroscopic demonstration that IMCL accumulation is associated with insulin resistance in children with prediabetes and that increased lipid content in myocytes is a marker of impaired insulin action.

Delineating mechanisms by which an increase in skeletal muscle lipid availability may confer insulin resistance in diabetes may help target specific pathways, proteins, or genes involved in insulin action. For example, excess triglyceride in insulin-resistant muscle might lead to elevated diacylglycerol (DAG) or fatty-acyl-coA concentration, which in turn activates a serine/threonine kinase cascade involving protein kinase C, leading to phosphorylation of serine/threonine sites in IRS-1 (23). Serine-phosphorylated forms of these proteins fail to associate with and activate PI3K, resulting in decreased activation of glucose transport (mainly GLUT-4) and other downstream associated events. If this hypothesis is correct, any perturbation that results in accumulation of intracellular fatty-acyl-coA or other fatty acid metabolites in muscle and liver, either through increased delivery and/or decreased metabolism, may be expected to induce insulin resistance and/or insulin action (23, 24). The determinants of the tendency to accumulate lipids within myocytes are strongly influenced by genetic and environmental factors.

Lipid droplets in the liver

Concurrent with the worldwide epidemic of childhood obesity, non-alcoholic fatty liver disease (NAFLD) has become the most common cause of unexplained abnormal liver function tests in the pediatric population (25) and may encompass the entire spectrum of liver conditions, ranging from simple steatohepatitis (NASH) to advanced fibrosis and cirrhosis. It affects 2.6% of children and 22.5– 52.8% of obese children (26). Schwimmer et al. (27) estimated that the prevalence of fatty liver is 9.6% in children aged 2 to 19 years in the county of San Diego, after adjusting for age, gender, race, and ethnicity. The highest rate of fatty liver was seen in obese children (38%). After controlling for the severity of obesity, Hispanic boys and girls have higher rates of fatty liver than non-Hispanic peers and they are more prone to advanced liver fibrosis. If the prevalence is similar for the entire United States, this would represent more than 6.5 million children and adolescents (27).

NAFLD, characterized by fatty infiltration of the liver in the absence of excessive alcohol consumption (28) and assessed from elevated serum liver enzymes, was found in the NHANES III survey to be more prevalent in obese African-American and Hispanic males with T2DM, hypertension, and hyperlipidemia (29). These

associations have led to the suggestion that NAFLD precedes the onset of diabetes in some individuals; indeed, a relationship between early alteration in glucose and lipid metabolism and the presence of hepatic steatosis in obese youth has been described (30). Insulin resistance was found to be a clinical correlate of alanine aminotransferase (ALT) elevations, a surrogate of hepatic steatosis, in small cohorts of obese Asian children (31, 32), and strongly associated with biopsy-proven steatosis (33) in a small group of mostly Hispanic and Caucasian boys. We have recently reported the metabolic consequences associated with elevations of ALT and hepatic steatosis in a large multiethnic cohort of obese children. We showed that elevations in ALT levels, even within the normal range, were associated with deterioration in insulin sensitivity and worsening glucose tolerance assessed by OGTT (34). Metabolic alterations were further exaggerated when ALT levels were elevated outside the normal range. Similar "dose–response" relationships were observed between rising ALT levels and increasing free fatty acid (FFA) and triglyceride levels. These relationships persisted after controlling for age, gender, ethnicity/race, and BMI z-score. In addition to demonstrating that insulin resistance is strongly and independently correlated with rising ALT levels, our study is the first to observe that early alterations in glucose tolerance are associated with rising ALT levels in obese children. It remains unclear whether intra-hepatic fat accumulation is a consequence or cause of the observed metabolic derangements in insulin sensitivity and lipid metabolism (elevated triglycerides and FFAs).

The noninvasive MRI technique, fast gradient echo magnetic resonance pulse sequences (fast-MRI), used in a random subset of subjects ($n = 72$) of our cohort more firmly established the connection between fatty liver disease and altered metabolism in obese children. In our subset of obese youth, steatosis was assessed as a hepatic fat fraction (HFF) $> 5.5\%$ (> 2.5 SD above the mean for our lean controls) and was associated with subtle changes in glucose tolerance, insulin resistance, rising triglyceride levels, and metabolic syndrome. In particular, 32% of our subjects had an HFF $> 5.5\%$, implying the presence of hepatic steatosis. Of note, only 48% of subjects with fatty liver had ALT level in the abnormal range. Obese adolescents with an HFF $> 5.5\%$ had a greater area under the curve (AUC) for glucose, and were significantly more insulin resistant (whole-body insulin sensitivity index, WBISI), as assessed by OGTT, compared to those with a low HFF. In addition, those with a high HFF had significantly higher triglyceride levels and significantly lower adiponectin levels. Furthermore, subjects with increased hepatic fat content tended to have a greater prevalence of IGT and had a significantly greater prevalence of metabolic syndrome. In obese children, NAFLD can be seen as another component or manifestation of metabolic syndrome (26, 1).

NAFLD is associated with increased visceral fat deposition in adults (36, 37) and in a recent small pediatric study (38). In our cohort, the abdominal visceral area, assessed by MRI technology, was found to be tightly linked with steatosis as assessed by fast-MRI. Indeed, the visceral fat depot was significantly greater in obese adolescents with a high HFF compared to those with a low HFF.

Intra-abdominal lipid accumulation has long been associated with insulin resistance and other components of the metabolic syndrome (39). Visceral fat is

less sensitive to insulin compared to subcutaneous fat, drains directly into the portal circulation, and seems to have a different adipocytokine secretion profile. Visceral adiposity has also been associated with greater peripheral insulin resistance (40), with a direct impact on insulin sensitivity and secretion, independent of total body adiposity (41). We have demonstrated that an altered distribution of fat between the subcutaneous and intra-abdominal compartment is associated with the development of IGT. The IGT subjects had relatively more visceral fat and less subcutaneous fat than NGT subjects. Therefore, the visceral-to-subcutaneous ratio was significantly greater in the IGT than in the NGT group. Both the enlarged visceral depot ($r = -0.63$, $p < 0.01$) and the visceral-to-subcutaneous ratio ($r = -0.66$, $p < 0.01$) were inversely related to the insulin-stimulated glucose metabolism (22). It appears that the ability of peripheral subcutaneous fat tissue to vary its storage capacity is critical for regulating insulin sensitivity and ultimately protecting against diabetes (Fig. 9.1).

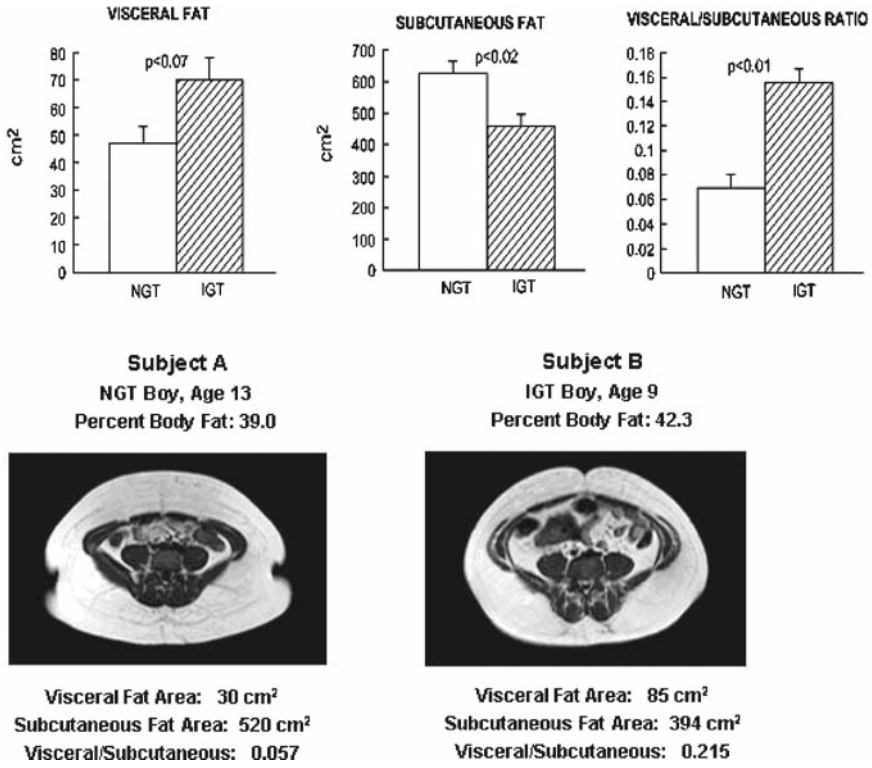

Fig. 9.1 Differences in visceral and subcutaneous abdominal fat and the visceral-to-subcutaneous ratio in obese children and adolescents with normal (NGT) and impaired glucose tolerance (IGT).

Transverse abdominal MRI scans (L4 vertebral level 1, fat appears white with T1 weighting) from an obese boy with NGT (Subject A) and one with IGT (Subject B). The subjects have equivalent amounts of total body fat, but there is more visceral fat and less subcutaneous fat in the subject with IGT (B) compared with the matched control (A)

We also found that variation in the composition of subcutaneous fat is linked to NAFLD. In particular, subjects with hepatic steatosis had significantly less subcutaneous fat and a higher deep-to-superficial subcutaneous fat ratio than those with an HFF <5.5% ($p = 0.026$, data not published). It became apparent that the overall lower amounts of subcutaneous fat in subjects with fatty liver were primarily a function of a decreased superficial subcutaneous fat.

Childhood obesity and inflammation

The coexistence of obesity and a low-grade inflammatory state has been found to be present during the earliest stage of obesity and is strongly dependent on the degree of obesity (42). One revolutionary concept is that the adipose tissue is not merely a simple reservoir of energy stored as triglycerides but also serves as an active secretory organ, releasing many peptides, complement factors, and cytokines into the circulation (43). In the presence of obesity, the balance between these numerous molecules is altered such that enlarged adipocytes and macrophages embedded within them produce more pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), and fewer antiinflammatory peptides, such as adiponectin. The dysregulated production of adipocytokines has been found to participate in the development of metabolic and vascular diseases related to obesity (44).

The role of adipocytokines in the development of altered glucose metabolism in children and adolescents is a novel and intriguing field of research. In adults, dysregulation of the expression and secretion of adiponectin may play a role in the pathogenesis of T2DM (45–48). In fact, studies in adults have shown that hypoadiponectinemia is an independent risk factor for the progression of T2DM (49–51).

Adiponectin is the only adipocytokine that is known to be inversely related to the degree of adiposity (46). We have recently shown that adiponectin levels differ between obese youth with NGT vs. IGT, despite a similar degree of obesity (46). In particular, we have shown that plasma adiponectin levels are positively related to whole-body insulin sensitivity, as assessed by the glucose clamp technique, whereas they are strongly inversely related to triglyceride levels, IMCL content, and fasting insulin levels. These relationships are independent of total body fat percentage and central adiposity. The close association with IMCL content is consistent with the data from Yamauchi et al. (47), indicating that adiponectin acts primarily on skeletal muscle tissue to increase influx and combustion of FFAs, thereby reducing muscle triglyceride content in the mouse model of obesity. Thus the modulatory effect of adiponectin on whole-body insulin sensitivity may be mediated, in part, via its effect on the IMCL content.

In an attempt to understand the relationship between obesity and inflammation, we analyzed the relationship between adiponectin and C-reactive protein (CRP), the prototype inflammatory marker, in a large multiethnic cohort of obese children and adolescents. Stratifying the cohort (589 obese children and adolescents) into

quartiles of serum adiponectin and adjusting for potential confounding variables, including age, gender, pubertal stage, ethnicity, BMI z-score, and WBISI, we found that low adiponectin levels are associated with higher CRP levels, and high density lipoprotein (HDL) cholesterol was the only component to show association with adiponectin. Hence, the relationship between adiponectin levels and both CRP and HDL cholesterol appear to be independent of obesity and insulin resistance in childhood obesity and are not influenced by ethnicity (p-value adjusted for ethnicity <0.003). One limitation of this analysis is the fact that we measured total adiponectin levels rather than the low and high molecular forms. However, it is likely that the high molecular form is more strongly related to insulin sensitivity than total adiponectin (48). Whether the antiinflammatory effects of adiponectin are also more closely related to its high molecular form remains unclear.

This study suggests that adiponectin could play a role in modulating CRP levels and therefore be a potential molecular link between adiposity and inflammation. However, this link may not be entirely due to its well-known effects on insulin sensitivity. Mechanistic studies are needed to understand whether the link is indeed real and, more importantly, how these various factors interact with one another during the development of the metabolic syndrome and cardiovascular disease.

Conclusion

Altered glucose metabolism in obese youth is an emerging phenomenon of the last two decades, strongly associated with the increase in the prevalence of childhood obesity. Childhood obesity sets the stage for multiple target organ damage and related morbidity. Prediabetes in obese children and adolescents represents a complex metabolic phenotype, characterized by peripheral insulin resistance and altered lipid partitioning. The combination of ectopic lipid deposition, an adverse adipocytokine profile, and possibly low-grade inflammation, may play a major role in the deterioration to overt diabetes. These events may proceed at an alarming rapid tempo and also play a role in the development of adverse cardiovascular outcomes related to the metabolic syndrome in these youngsters. Primary prevention of childhood obesity and tailored conservative and/or pharmacologic interventions for obese youth with prediabetic conditions hold the promise of halting the rising prevalence of T2DM in this pediatric age group.

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Chapter 10
Mediators of Insulin Resistance

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Keywords insulin resistance, free fatty acids, metabolic syndrome, adipokine, adiponectin, leptin, TNF-α, RBP-4, resistin, visfatin

Introduction

Metabolic syndrome (MS) is characterized by resistance to insulin action on skeletal muscle, liver, and fat. Though the pathophysiology of insulin resistance in human disease is still poorly understood, a number of circulating factors that modulate insulin action have now been identified. Most of these factors are secreted by adipose tissue, and indeed, adipose tissue is now recognized to be an endocrine organ, with a central role in the regulation of insulin action and whole-body glucose and lipid metabolism. Adipose tissue dysfunction associated with obesity is believed to be an underlying defect in the development of insulin resistance accompanying MS.

One of the main actions of adipose tissue is to regulate free fatty acid (FFA) flux. FFAs are stored in adipose tissue in the form of triglycerides, and released into the circulation through the process of lipolysis. In addition to controlling FFA metabolism, adipose tissue secretes a number of proteins (so-called adipokines) into the circulation. FFAs and adipokines can affect insulin signaling and modulate whole-body glucose metabolism. Adipose tissue dysfunction associated with obesity causes a dysregulation of FFAs and adipokines, resulting in abnormal circulating levels of these products.

Insulin action occurs through the binding of insulin to its cell-surface receptor in target organs. This binding results in the autophosphorylation of the insulin receptor and initiates the activation of intracellular signaling cascades, which in turn are responsible for insulin's metabolic and mitogenic effects. In the setting of insulin resistance, insulin signaling is impaired, and higher than normal levels of insulin are needed for normal insulin response. Impaired insulin action results in a disinhibition of lipolysis in adipose tissue, impaired glucose utilization by muscle, and disinhibition of gluconeogenesis by the liver. As a consequence, hypertriglyceridemia and hyperglycemia develop.

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Circulating FFAs and adipokines interact with components of the insulin signaling mechanism at target tissues, thereby modulating insulin action. Abnormal circulating levels of adipose tissue products resulting from obesity-induced adipose tissue dysfunction are therefore believed to be involved in the pathogenesis of insulin resistance in the MS. A great deal of research has now been focused on the differential function of visceral vs. subcutaneous adipose tissue. Though the functional profiles of these two adipose depots do appear to differ somewhat, the contribution of each depot to the development of whole-body insulin resistance is still under investigation.

This chapter will review the current evidence for the role of adipocyte products as humoral mediators of insulin resistance.

Free fatty acids (FFAs)

In healthy individuals, adipose tissue takes up and stores FFAs during times of energy excess, and releases FFAs to be used as fuel by the skeletal muscle, the heart, and the liver during periods of low energy intake or fasting. With prolonged periods of excess energy intake and enhanced fat stores, circulating FFAs are not adequately cleared and FFA release is not inhibited despite adequate fuel availability. Under these conditions, plasma FFA levels remain chronically elevated, and lipid storage occurs in non-adipose tissues, contributing to skeletal muscle and liver insulin resistance.

Elevated plasma FFA levels have been reported in individuals with uncontrolled diabetes (1) as well as in non-diabetic insulin-resistant individuals, such as those with obesity or a family history of type 2 diabetes (2). In addition, FFA concentrations have been shown to correlate with skeletal muscle insulin resistance (2). In non-diabetic subjects, acute elevation of circulating FFAs has been reported to cause insulin resistance within 2–4 h, with normalization of insulin action several hours after normalization of plasma FFA levels (3, 4).

Visceral adipose tissue has higher rates of lipolytic activity than subcutaneous adipose tissue, making it a likely candidate for FFA-induced insulin resistance (5). However, recent studies in vivo have reported that the upper-body subcutaneous adipose tissue, not the visceral tissue, is the main source of FFAs in both lean and obese subjects after an overnight fast (6). Multiple mechanisms for the induction of insulin resistance by FFA have now been proposed.

Effect of FFAs on skeletal muscle insulin action

The main action of insulin on skeletal muscle is to regulate glucose utilization. Competition between FFA oxidation and glucose oxidation results in decreased glucose uptake and utilization by muscle in the setting of excess FFA availability.

This glucose–fatty acid cycle theory, initially proposed by Randle et al. in 1963 (7), could explain FFA-induced insulin resistance in the muscle. However, more recent observations, reviewed by Roden, have led to the proposal of other mechanisms to explain this effect (8).

Current data suggest that FFAs induce insulin resistance in human skeletal muscle by impairing the insulin signaling pathway (9, 10). Elevated plasma FFA levels result in intramyocellular triglyceride storage (11, 12), with an associated accumulation of intramyocellular lipid metabolites (13). These lipid metabolites (acyl-CoA, ceramides, diacylglycerol) activate several serine/threonine (Ser/Thr) kinases (14), such as protein kinase C (PKC), inhibitory kinase β (κB) and nuclear factor κB (NF-κB). These Ser/Thr kinases inhibit insulin signaling by phosphorylating several proteins in the insulin-signaling cascade, resulting in decreased insulin-stimulated glucose transport (GLUT 4) activity and decreased glucose uptake (13). FFAs can also cause insulin resistance through the production of reactive oxygen species (ROS) (15). ROS can activate PKC and NF-κB, again resulting in Ser/Thr kinase inhibition of the insulin signaling cascade (16, 17).

Effect of FFAs on liver

The main action of insulin in the liver is to regulate glucose production. FFAs increase hepatic glucose production by inhibiting insulin-mediated suppression of glycogenolysis (18). This effect of FFAs likely occurs through similar intracellular mechanisms as those described in skeletal muscle (14).

In addition to inhibiting insulin action, FFAs also stimulate gluconeogenesis through the formation of acyl-CoA and subsequent production of intermediate substrates in the gluconeogenic pathway. In healthy subjects, hepatic autoregulation prevents this FFA-induced increase in gluconeogenesis from increasing overall hepatic glucose production (19). However, in patients with type 2 diabetes, hepatic autoregulation is impaired and FFA elevations do result in increased hepatic glucose production, contributing to hyperglycemia (20).

Effect of FFAs on adipose tissue

The effect of FFAs on adipose tissue has been studied in animal models, but to our knowledge, no human studies have yet been published. As with skeletal muscle and liver, elevated plasma FFAs inhibit insulin signaling in rodent adipose tissue through Ser/Thr kinase phosphorylation of intracellular insulin signaling proteins (21, 22).

The main action of insulin in adipose tissue is to suppress lipolysis, resulting in a decrease in FFA release into the circulation. Therefore, FFA-mediated insulin resistance in adipose tissue would be expected to result in impaired insulin-stimulated

suppression of lipolysis and a further increase in FFA secretion. In addition, adipose tissue produces multiple cytokines that affect whole-body glucose metabolism. In rodent studies, FFAs have been reported to increase the secretion of the insulin resistance inducer TNF-α, and to decrease secretion of insulin-sensitizing adiponectin (22).

Adiponectin

Adiponectin is a 30-kD, collagen-like protein synthesized by adipocytes, which circulates in human plasma as approximately 0.01% of total plasma protein (23). Adiponectin is lower in males than females and in patients with type 2 diabetes, and correlates negatively with intra-abdominal fat, age, body mass index (BMI), insulin resistance and plasma insulin, triglyceride, glucose, and C-reactive protein (CRP) levels (24–31). Conversely, adiponectin is positively correlated with high density lipoprotein (HDL) cholesterol (24) and is increased in type 1 diabetes and in persons with decreasing renal function (32–38). A beneficial effect of adiponectin on the development of atherosclerosis has been reported (39–41).

Published research on adiponectin has increased dramatically in the past decade, though space limitations preclude a comprehensive review of the literature. However, numerous reviews on adiponectin including its relationship to insulin resistance, MS, type 2 diabetes, and cardiovascular disease have been published (42–49).

Adiponectin and Insulin Resistance

Adiponectin is inversely correlated with insulin resistance, abdominal fat, and triglycerides and positively correlated with HDL cholesterol (31). Hypoadiponectinemia is related to insulin resistance and hyperinsulinemia (25) and precedes diminished insulin sensitivity (50). Higher adiponectin levels predict improved insulin sensitivity and affects insulin-stimulated glucose disposal and lipoprotein metabolism, including post-prandial FFA release (51). Adiponectin has been shown to improve insulin sensitivity in mice by decreasing triglycerides in the liver and muscle of obese mice, and physiologic doses of adiponectin and leptin normalized insulin resistance in lipoatrophic mice (52). Adiponectin correlates positively with glucose disposal rate and nonoxidative glucose disposal rate, but not fat oxidation, suggesting that adiponectin affects insulin sensitivity via skeletal muscle glycogen synthesis (53).

In addition to insulin sensitivity, low levels of adiponectin are related to type 2 diabetes. In rhesus monkeys, adiponectin decreased with development of obesity, and this decrease was in parallel with progression of insulin resistance (54). In case–control studies in both Pima Indians and the EPIC study, higher levels of

adiponectin were protective against development of type 2 diabetes (55, 56) and, similarly, a low level of adiponectin was an independent predictor of type 2 diabetes in Japanese (57) and Asian Indian populations (58). Adiponectin has also been reported to be lower in first-degree relatives of type 2 diabetes patients than matched controls (59).

Adiponectin and Vascular Disease

In addition to a beneficial association with insulin sensitivity and the development of type 2 diabetes, adiponectin has a protective effect on the development of cardiovascular disease, the leading cause of death in people with diabetes. Adiponectin accumulates in damaged vascular walls (60) and beneficially modulates the endothelial inflammatory response to vascular injury (61, 62). In cross-sectional data, adiponectin has been hypothesized to be increased as a compensatory response in type 1 diabetes patients with microvascular complications (35–37). The reasons for elevated adiponectin in type 1 diabetes and the paradox between the known anti-atherogenic effects of adiponectin and the premature mortality from coronary artery disease in type 1 as compared to people without DM are unclear (63). Hypotheses to explain elevated adiponectin in type 1 diabetes include the following: a compensatory response to vascular injury (35, 36), decreased clearance due to renal insufficiency (64), effects of subcutaneous insulin treatment (65), and post-translational modifications (glycosylation) (66, 67) that could differ in people with type 1 diabetes.

Data *in vitro* suggest that adiponectin has a beneficial role in modulating inflammatory response by inhibiting expression of adhesion molecules on endothelial cells (61), by inhibiting NF-κB signaling, which is an important pathway in endothelial function (68), and by suppressing macrophage activity (69). Additionally, adiponectin localizes to areas of vascular injury (60) and beneficially inhibits growth factors involved in neo-intimal thickening and smooth muscle cell proliferation (70). Low levels of adiponectin in vivo have been associated with high levels of inflammatory markers, such as CRP and IL-6 (71, 72) as well as IL-8 and TNF-α (73). Adiponectin has also been associated directly with lipoprotein lipase activity (74) and inversely with plasma hepatic lipase activity (which may link adiponectin to HDL) (75). Given adiponectin's relationship with obesity and insulin resistance, as well as other cardiovascular risk factors, it has been proposed as a link in the adipose–vascular axis (70) and to play a protective role in the development of atherosclerosis (39–41).

Adiponectin in Youth

In addition to data in animals and adults, adiponectin has been investigated extensively in youth. In a study of Pima Indian children, adiponectin concentration was inversely related to adiposity and longitudinally decreased with increasing

adiposity (76). Obese children with impaired glucose tolerance had lower levels of adiponectin than normal controls (77), and the data of Caprio and colleagues further suggests that intramyocellular lipid content may mediate the relationship between adiponectin and insulin sensitivity (77). Systolic blood pressure, another component of MS, was also inversely related to adiponectin in healthy adolescent Asian females (78). An additional report in children correlates adiponectin with fat mass, insulin sensitivity, and cardiovascular risk factors as in adults (79). The relationship of adiponectin to leptin has been reported to help discriminate type 1 from type 2 diabetes in youth (80), which is often a challenge with increasing rates of obesity.

While adiponectin is higher in adult women than men, the study of the development of this sex discordance indicates a distinct effect of pubertal maturation. In cross-sectional data, adiponectin was lower during puberty in boys than girls and was inversely correlated with testosterone; adiponectin was also inversely correlated with insulin resistance and obesity in both boys and girls (81). Adiponectin significantly declined with pubertal development in healthy, lean boys and was inversely correlated with both testosterone and DHEAS, with pubertal stage being the strongest independent predictor of adiponectin concentration (82).

In obese children, adiponectin was closely associated with MS parameters including insulin resistance (82). Other studies have suggested that age- and sex-related adiponectin levels are complex, with sex-discordant associations between adiponectin and lipid profiles (83). Higher adiponectin levels also attenuated the relationship between markers of insulin resistance and adiposity (84). With increasing adiposity, adiponectin's relationship with insulin and lipids strengthens, and therefore higher levels of adiponectin may have a greater benefit in obese than lean youth (85). Cross-sectional data in a large multiethnic cohort of obese children and adolescents suggest that adiponectin may be a biomarker of inflammation and MS in childhood obesity (86).

A physiologic change in insulin sensitivity during puberty related to increases in growth hormone and sex steroid hormones has been well described (87, 88). Recently, in a small longitudinal insulin clamp study, a 50% decrease in adiponectin levels was reported with puberty, which was in proportion to decreased pubertal insulin sensitivity (89).

In a hyperinsulinemic-euglycemic clamp study in African-American and Caucasian youth, adiponectin was an important independent predictor of insulin sensitivity and explained 27% of the variance in insulin sensitivity. Furthermore, racial differences in insulin sensitivity disappeared after adjustment for adiponectin, suggesting that adiponectin is an important marker of insulin sensitivity, and that lower adiponectin levels in African–American youth may place them at greater risk of insulin resistance, despite the presence of lower visceral fat than Caucasians (90). Lower adiponectin levels in African–American children compared to Caucasian children had been reported previously; adiponectin was positively correlated with insulin sensitivity, although adiponectin did not predict change in insulin sensitivity over 2 years of follow-up (91).

Although adiponectin has been shown to have a beneficial effect on atherosclerosis in adults (39, 40, 92), adiponectin was not associated with brachial-artery-flow-mediated, endothelial-dependent vasodilation and distensibility in adolescents cross-sectionally, although it was correlated with insulin resistance estimated by HOMA (93).

Mechanism of action

Adiponectin is a complex molecule and many questions still remain regarding the mechanism of its insulin sensitizing actions. One difficulty in interpreting studies of adiponectin action relates to the various adiponectin ligands used by different labs. Some labs have looked at the effect of a bacterially produced globular fragment of the protein, while others have used a full-length molecule derived from bacteria or mammalian cells. The globular fragment seems to be the most active and has been suggested to be the active form of the protein (94), yet to date it has not been detected in vivo. Another complication is that adiponectin exists as a supramolecular structure that forms trimers, hexamers, 12mers, and 18mers. The multimeric form or forms responsible for adiponectin's physiological actions have not yet been identified, though some studies suggest that the high molecular weight (HMW) forms comprising 12mers and 18mers and above may be more important in determining insulin sensitivity. While the HMW isoform of adiponectin is considered the biologically active form of the protein (95), different commercially available assays of total and HMW adiponectin have been reported to have varying relationships to insulin sensitivity (96). Mammalian full-length adiponectin with the ability to form higher-order multimers has been produced and its effect studied in mice (97).

The effect of acutely increasing circulating adiponectin on glucose metabolism has now been reported by several groups. Intraperitoneal injection of purified full-length adiponectin produced in a mammalian expression system (97) resulted in a significant drop in serum glucose concentrations in fasting wild-type, obese diabetic (ob/ob), and non-obese diabetic (NOD) mice. This decrease in glucose was attributed to enhanced suppression of hepatic glucose production and was not associated with increased insulin secretion. The same investigators demonstrated improved insulin-mediated suppression of hepatic glucose production in isolated rat hepatocytes treated with adiponectin. In obese mice, adiponectin treatment has also been shown to reverse insulin resistance by decreasing triglyceride content in muscle and liver (52).

Adiponectin's insulin-sensitizing actions have been attributed to the activation of AMP kinase (AMPK) (98, 99) by adiponectin receptor 1 (AdipoR1) (100), leading to increased expression of proteins involved in fatty-acid oxidation in muscle (94). AMPK stimulation in vivo and in vitro by adiponectin has been demonstrated in both skeletal muscle and the liver (98). The HMW form of adiponectin also seems to activate the NF-κB pathway in vascular muscle and endothelial cells (101), though the implications of this action on changes in insulin sensitivity remain speculative.

In addition to its insulin-sensitizing actions, adiponectin has been reported to have antiatherogenic effects, to alleviate alcoholic and non-alcoholic fatty liver disease, and to increase insulin secretion by pancreatic islet cells. The molecular mechanism of these additional actions of adiponectin will not be discussed here.

Therapeutic Effects on Adiponectin

Given adiponectin's beneficial effect on insulin resistance and cardiovascular disease, and the increasing mechanistic understanding of its role in these processes, therapeutic interventions including both therapeutic lifestyle change and medications that increase adiponectin are of interest. Weight loss has been shown to increase adiponectin levels in both type 2 and non-diabetic subjects (24). The insulin-sensitizing medication class thiazolidinediones (TZDs) has also been shown to increase adiponectin levels in mouse models (52) as well as in both diabetic and non-diabetic subjects (102, 103). Furthermore, Scherer and colleagues have shown that TZDs cause a shift in adiponectin complex distribution (increased HMW adiponectin) and that this increased high to low molecular weight ratio may explain the improvement in insulin sensitivity with TZD usage (104). Additionally, other medications commonly used to block the renin angiotensin system angiotensin-converting enzyme inhibitors; Angiotensin receptor blockers have been reported to increase adiponectin levels (105, 106). A thorough review of the effect of therapeutic interventions on adiponectin levels (with emphasis on cardiovascular disease) has been published recently (49).

Leptin

Leptin is a cytokine that is synthesized and released into the circulation by adipocytes. Leptin's main action is on the hypothalamus, where it regulates food intake and energy expenditure via the activation of anorexigenic peptides and the inhibition of orexigenic peptides. In addition to its central effects, leptin has been shown to have direct peripheral effects, activating AMP-dependent protein kinase in muscle, and resulting in activation of fatty acid oxidation (107).

In humans, leptin deficiency and leptin receptor mutations have been reported to cause severe obesity (108, 109). Leptin administration reverses insulin resistance in lipodystrophic animals (110) and in obese humans with low leptin levels (111). Children with leptin deficiency treated with leptin lose weight, resulting in improved insulin sensitivity (109). However, most cases of human obesity are associated with high leptin levels, suggesting a leptin-resistant state (112). The molecular basis for this leptin-resistant state is not yet understood. Though mutations in the leptin receptor gene have been described (113), these appear to be rare in humans. In addition, leptin administration to a group of obese humans resulted in modest weight loss, without any changes in glycemic control or insulin action (114). Thus, the role of leptin resistance in mediating insulin resistance in human obesity remains unclear.

Tumor Necrosis Factor α (TNF-α)

TNF-α is a cytokine produced by a number of different cell types, including adipocytes and macrophages. This cytokine was first proposed as a link between obesity and insulin resistance in 1993, when elevated concentrations of TNF-α mRNA were demonstrated in adipose tissue from four different rodent models of obesity and diabetes, and neutralization of TNF-α in obese rats was shown to improve insulin resistance (115). TNF-α expression has also been shown to be elevated in obese humans and to decrease with weight loss (116).

There are two main mechanisms through which TNF-α is thought to cause insulin resistance. First, it has been shown to cause serine phosphorylation of IRS-1 (117), inhibiting insulin signaling, much like FFAs. Second, TNF-α stimulates adipose tissue lipolysis (118), resulting in increased circulating FFA levels, which in turn lead to insulin resistance through mechanisms previously discussed. In addition, TNF-α may increase adipose tissue inflammation by stimulating monocyte chemotactic protein 1 and increasing recruitment of macrophages into the adipose tissue (119).

Retinol-Binding Protein 4

Retinol-binding protein 4 (RBP4) is secreted by adipocytes and is correlated with insulin resistance in adults (120) and healthy adolescents (121). Change in RBP4 in response to exercise training predicted the improvement in insulin sensitivity better than other adipokines or inflammatory markers, and a mechanistic link between reduced GLUT4 protein in adipocytes, elevated RBP4, and insulin resistance has been hypothesized (120). Lowering RBP4 levels to improve insulin sensitivity has been suggested as a possible therapeutic strategy (122). The inverse relationship between RBP4 and insulin sensitivity has been demonstrated in non-diabetic adults (123), and RBP4 is elevated in adults with impaired glucose tolerance (124). Conversely, a more recent paper reports that RBP4 was not significantly associated with insulin sensitivity or obesity but instead had a significant association with insulin secretion (as measured by the insulin disposition index) (125).

Other adipokines

Resistin

The role of resistin in human insulin resistance remains unclear. Resistin was initially described in mice (126), and has been reported to be increased in murine models of obesity (127). In addition, antiresistin antibodies have been shown to improve insulin sensitivity in obese mice (128). In humans, resistin is mostly made

by circulating monocytes (129), and resistin expression from adipocytes does not seem to correlate with insulin resistance or obesity (129–131).

Visfatin

Visfatin is a recently isolated adipokine, initially reported to be produced mainly by visceral adipose tissue (132). Fukuhara et al. identified visfatin by using a cDNA library derived from two female volunteers and looking for genes more highly expressed in visceral than subcutaneous fat. They then demonstrated a correlation between plasma visfatin levels and visceral adipose tissue mass in 101 human subjects from Japan. However, a subsequent study of a German population done by Berndt et al. (133) found no association between plasma visfatin levels and visceral adiposity.

Visfatin has been shown to have insulin-mimetic effects on cultured cells as well as in mice in vivo (132). In addition, it has been shown to bind to the insulin receptor and activate insulin signaling cascades (132). However, Berndt et al. reported no association between plasma visfatin levels and parameters of insulin sensitivity.

In humans, plasma visfatin levels are very low, and the impact of this protein on whole-body insulin sensitivity remains in question.

Conclusion

Adipose tissue dysfunction, resulting in abnormalities in FFA flux and adipokine production and secretion, has been identified as a likely mechanism for insulin resistance. Adipose tissue products such as FFAs, adiponectin, and TNFα have been shown to modulate insulin action through their effects on intracellular insulin-signaling pathways. Adiponectin, found in high concentrations in human plasma, but decreased in MS, has been of particular interest owing to its insulin-sensitizing and anti-atherogenic effects. Much research remains to be done to understand the true role of adipokines in mediating insulin resistance in human disease.

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Chapter 11
Insulin Resistance and the Pathogenesis of Cardiovascular Disease

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Keywords atherosclerosis, thrombosis, coronary artery disease, inflammation, endothelial dysfunction, insulin resistance

Introduction

The prevalence of diabetes has considerably increased over the past few decades, and is predicted to reach 4.4% worldwide by 2030 (1). Hyperglycemia in diabetes is due to a combination of decreased tissue sensitivity to insulin, increased hepatic production of glucose, and impaired insulin secretion by the pancreas. Insulin resistance (IR) is a state that requires increased concentrations of insulin to achieve its desired biological effect and is tightly linked to excess white adipose tissue and obesity (2). In the early stages of IR, hyperinsulinemia takes place through additional insulin production by the pancreas, to compensate for "decreased insulin activity", thereby keeping the blood glucose within the normal range. Without intervention to restore normal insulin sensitivity, the pancreas eventually loses its capacity to produce extra insulin (secondary β-cell failure) and the ensuing hyperglycemia results in the development of type 2 diabetes (T2DM).

The link between obesity and diabetes is clearly established in both men and women (3, 4). The current global epidemic of obesity (exceeding 30% in some American states) contributes to the increased prevalence of diabetes and there has been an alarming increase in childhood and adolescent obesity and IR in recent years (5, 6), leading to the development of diabetes at a relatively young age. The increasing prevalence of obesity, IR, and diabetes in children is frequently associated with an unhealthy diet and lack of physical exercise (7).

IR plays a role in predisposition to diabetes and is an important risk factor for atherothrombotic disease.

The association between central obesity and vascular disease was observed in the first half of the 20th century (8) and this was followed a quarter of a century later by the documentation of a link between IR and myocardial infarction (MI) (9). The association between diabetes and cardiovascular disease (CVD) is now well documented (10), and studies have shown a 20% increase in cardiac death in diabetic patients over a period of

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7 years, which further increases to 45% in the presence of a previous history of myocardial infarction (11). Moreover, in the prediabetes state (impaired fasting glucose or impaired glucose tolerance), subjects are also at a higher risk of atherothrombotic events compared with the general population (12). Equally, most non-diabetic patients with recent MI have evidence of impairment in glycaemic control (13).

The close association between T2DM and CVD led to the suggestion that these two clinical entities represent the same clinical condition underpinned by a number of risk factors, which are closely associated with IR (The Common Soil Hypothesis). These risk factors can be divided into traditional – including hypertension, dyslipidemia, microalbuminuria, and deranged glucose metabolism – and non-traditional, which include low-grade inflammation, endothelial dysfunction, and hypercoagulation. This clustering of risk factors can be seen at a very early age, as childhood obesity is associated with IR, impaired glucose tolerance, hypertension, and platelet activation, indicating the early presence of cardiovascular abnormalities in obese children (14, 15). It has recently been proposed that IR is the primary biochemical defect underlying the current epidemic of obesity, CVD, and T2DM (16, 17). The mechanism by which IR predisposes to diabetes may be straightforward (detailed above), but the mechanisms involved in predisposition to atherothrombotic disease are more complex.

The current chapter will review the role of IR in predisposition to atherothrombotic disease and will analyze some of the mechanisms involved. The role of improving insulin sensitivity in the prevention and treatment of diabetes and atherothrombotic disease will also be discussed.

Pathogenesis of Atherothrombosis

Studies have shown that vascular changes can be detected in young, overweight subjects, indicating that the pathogenic process starts at an early age (18, 19). The pathogenic mechanisms in atherothrombosis are complex and involve multiple pathways. The earliest abnormality is endothelial dysfunction, characterized by a decrease in barrier function of the endothelial cells (EC), coupled with impairment in secretory capacity (essential for vascular homeostasis). A local and generalized inflammatory reaction further contributes to the development of atherosclerosis, both early and late in the disease process. In the later stages of the disease, hypercoagulation and hypofibrinolysis play a role in local thrombus formation in the diseased artery, resulting in vessel occlusion, which manifests clinically as MI, stroke, or limb ischemia. The role of endothelial dysfunction, low-grade inflammation, and hypercoagulation in the pathogenesis of CVD is discussed in detail below.

Endothelial dysfunction

Compelling evidence supports endothelial dysfunction as a key, early event in the pathogenesis of atherosclerosis (20). The term endothelial dysfunction encompasses several potential abnormalities, the most extensively studied being a reduction in the

bioavailability of nitric oxide (NO). NO is an antiatherosclerotic signaling molecule released by the vascular endothelium that has potent vasodilatory (21, 22), antiinflammatory (23, 24), antiproliferative (25–28), antioxidant (29, 30), and antiplatelet (31, 32) effects. A reduction in NO bioavailability is present in atherosclerotic vessels before vascular structural changes occur. Consonant with this, longitudinal studies have shown that impaired NO-dependent vasodilatation is a predictor of future cardiac events (32) and the development of coronary artery atherosclerosis (33).

Insulin resistance and endothelial dysfunction

Obesity is characterized by endothelial dysfunction (see (34) for review), providing at least one putative mechanistic link with future atherosclerosis. In obese non-diabetic humans, Steinberg et al. (35) demonstrated that the increase in blood flow into the leg in response to methacholine, an endothelium-dependent muscarinic agonist, is blunted, with the abnormality being proportional to the degree of obesity. Laine et al. (36) showed that the ED50 for bradykinin to increase leg blood flow is double in obese subjects compared to lean ones. NO production in response to Ca_2^+-independent stimuli is also abnormal in obese humans. Arcaro et al. showed that the blood flow response to shear stress is blunted in obese subjects (37). Likewise, Tack et al. demonstrated that the forearm vasodilatory response to insulin is blunted (38), and Westerbacka et al. (39) confirmed a similar response in large vessels.

In otherwise healthy subjects, we have shown that obesity is characterized by abnormalities of metabolic and blood pressure homeostasis, heightened systemic inflammation, insulin resistance, and significant impairment of shear-stress-induced changes in forearm conduit artery blood flow (40). We correlated endothelial function with these abnormalities and found that blood pressure, inflammatory markers, serum insulin, and lipids were all negative correlates of impaired endothelial function (41), a finding that illustrates the complexity of the potential mechanisms of endothelial dysfunction in obese humans. In young insulin-resistant Asian men compared to young Caucasian men, we recently found that conduit artery endothelial function is blunted. Moreover, insulin-resistant Asian men have a reduction in the number and function of circulating endothelial progenitor cells (EPCs) (42), which have been suggested to play an important role in endothelial repair (43).

Endothelial dysfunction has been demonstrated in several non-diabetic animal models of obesity/insulin resistance. Winters et al. demonstrated blunting of the vasorelaxant effect of acetylcholine in aortic rings of leptin-deficient ob/ob mice (44). In Zucker fatty rats, endothelial dysfunction and blunting of insulin-mediated activation of endothelial NO synthase (eNOS) (45) have been demonstrated. More recently, we also demonstrated that both insulin- and acetylcholine-mediated NO release are blunted in a feeding model of obesity in mice (46).

NO is generated by a family of nitric oxide synthases (NOSs) from L-arginine in a reaction that requires oxygen, NADPH, and the essential co-factors tetrahydrobiopterin (BH4), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN). NO production and bioavailability are regulated/dysregulated at several levels.

Expression of eNOS

Although eNOS is constitutively expressed, studies, both in vivo and in vitro, have established that eNOS expression is subject to regulation by several (patho-) physiological factors, including insulin (47), shear stress (48), and cytokines (49). Consistent with a role for insulin to upregulate eNOS transcription, we have demonstrated that non-diabetic mice with hyperinsulinemia within the physiological range have increased eNOS mRNA in aortic tissue and functional evidence of increased NO production (50). In contrast to this, in mice rendered obese by a high fat diet, we found no change in eNOS mRNA but a significant increase in inducible NOS mRNA in keeping with low-grade vascular inflammation (46).

Abnormalities of agonist-mediated NO release

The activation of eNOS occurs through a number of distinct intracellular signaling pathways that may be affected in disease states. In very early atherosclerosis, there is reduced responsiveness to receptor-dependent stimuli, such as acetylcholine, whereas responsiveness to receptor-independent stimuli, such as the calcium ionophore A23187, is unchanged (51). These data indicate that in this setting there is blunting of NO production in response to extracellular stimuli, whereas eNOS expression and potential maximal activation are unaffected. In mice heterozygous for a knockout for the insulin receptor (IRKO), we have demonstrated an age-related decline in endothelial function, with preserved relaxation to acetylcholine in young mice and blunted responses in older mice (52). The mechanisms underlying this still require investigation.

Calcium-dependent and -independent eNOS activation

Classical activation of eNOS (e.g., by acetylcholine) involves a rise in intracellular Ca^{2+} and binding of Ca^{2+}/calmodulin to the enzyme. Recently, a Ca^{2+}-independent regulatory pathway for eNOS has been described (53), which may be of particular relevance to obesity. Both shear stress and agonists such as insulin have been shown to increase endothelial NO production via activation of phosphatidylinositol 3-kinase (PI3K) and protein kinase-B (PKB/Akt), which phosphorylates eNOS (discussed in detail below). In wild-type mice, we have shown insulin-mediated vasorelaxation to be endothelial, NO, and PI3K dependent (54).

Inactivation of NO by reactive oxygen species (ROS)

The evidence that production of ROS, such as superoxide, within the vascular wall plays an important role in the development of endothelial dysfunction is compelling (55). Superoxide leads to endothelial and vascular dysfunction in several ways:

(a) it reacts rapidly with NO to inactivate it (56); (b) the reaction between NO and superoxide produces peroxynitrite, which may itself exert toxic effects through protein nitrosylation (57); (c) species such as H_2O_2 and peroxynitrite (and the loss of NO) may activate redox signaling cascades that induce deleterious changes in endothelial cell phenotype. In a murine model of obesity (without diabetes), we have recently demonstrated that although basal ROS production in aortae is similar in lean and obese mice, in response to acetylcholine there is a substantial release of H_2O_2 (46). There are several potential sources of ROS in the vascular wall, such as xanthine oxidase, mitochondria, dysfunctional NOSs, and the phagocyte-type NADPH oxidase(s), that have recently emerged as major sources of superoxide in the vasculature (for review see (58)) and in adipose tissue of obese mice (59).

Alteration of tetrahydrobiopterin (BH_4) availability

BH_4 is a critical cofactor for eNOS activation. The rate-limiting enzyme in BH_4 biosynthesis is GTP cyclohydrolase-1 (GTPCH1). Importantly, in the setting of BH_4 deficiency, eNOS can generate superoxide rather than NO (60). Furthermore, BH_4 itself is rapidly degraded by ROS such as superoxide, so that a vicious cycle can ensue whereby oxidative stress worsens BH_4 deficiency. Recent data have shown that BH_4 deficiency is an important mechanism for endothelial dysfunction, and that the administration of BH_4 (or sepiapterin, which increases BH_4 production) can improve endothelial function in fructose-fed diabetic rats (61). However, its role in IR and obesity (prediabetes) is unclear. There is some evidence to support a role for insulin in upregulation of GTPCH1 activity, an effect blocked by wortmannin, an inhibitor of PI3-kinase (62). The effect of changes in insulin signaling on GTPCH1 activity is incompletely explored in vivo. Recent studies of mice with endothelial-cell-specific overexpression of GTPCH1 demonstrated that these mice had enhanced eNOS activity and were protected against endothelial dysfunction in a model of (insulin deficient) type 1 diabetes (63).

Inflammation

Inflammation, plaque formation, and rupture

Local vessel inflammation and a generalized inflammatory response seem to play a role in the atherothrombotic process. Early in the disease, dysfunctional EC express adhesion molecules that promote attachment of monocytes to the EC. Migration of adherent monocytes through the vessel wall is facilitated by monocyte chemoattractant protein-1 (MCP-1), and these cells subsequently undergo transformation into macrophages. At the same time, the barrier function of diseased EC is compromised and low-density lipoprotein cholesterol (LDL) is leaked into the vessel wall, where it is modified to oxidized (ox)-LDL, a powerful pro-atherogenic

molecule. Migrating macrophages take up ox-LDL by binding to CD36 and transform into foam cells, which further enhance the atherogenic process by secreting inflammatory cytokines, such as interleukin (IL)-1 and IL-6, and chemokines such as MCP-1 (64). The production of inflammatory mediators by foam cells draws in more inflammatory cells into the vessel wall, including T cells, which through the secretion of a variety of cytokines, results in augmentation of the inflammatory reaction, proliferation of vascular smooth muscle cells (VSMC), and production of proteoglycans, consequently contributing to expansion of the extracellular matrix (65). Additionally, foam cells migrate into the subendothelial space to form the fatty streak, which represents the earliest visible lesion in the atherosclerotic process, and collagen forms around the fatty streak to generate the atherosclerotic plaque. Inflammatory molecules, including cytokines and macrophage-produced matrix metalloproteinases (MMP), degrade collagen, rendering the atherosclerotic plaque prone to rupture (66). Once the plaque ruptures, it permits contact between the procoagulant lipid core and blood, which initiates thrombus formation (detailed below). The importance of these early processes in the generation of atherothrombotic disorders is evidenced by the identification of fatty streaks in young children and particularly in those who develop obesity early in life.

CD40/CD40 ligand (L) system

The expression of CD40L can be induced by inflammatory molecules on a variety of cells including EC, smooth muscle vascular cells, and platelets (67–69). The interaction between CD40L and CD40, the latter being constitutively expressed on numerous cells (70), results in the production of inflammatory molecules as well as growth and procoagulant factors, suggesting a key role for CD40L in disease progression and thrombus formation. Soluble (s) plasma CD40L levels, which are predictive of adverse outcome in patients with coronary artery disease (71, 72), are elevated in obesity and correlate with IR (73). Furthermore, weight loss has been shown to be associated with a reduction in sCD40 levels coupled with an improvement in IR (73, 74).

Oxidative stress

Oxidative stress results in the production of ROS that can be detected in atherosclerotic vessels, and have been shown to catalyze the formation of ox-LDL, an essential early step in atheroma formation (75). Later in the disease process, ROS weaken the atherosclerotic plaque, making it susceptible to rupture (76). IR is associated with increased oxidative stress, suggesting one mechanism for increased cardiovascular risk in patients with diabetes (59, 77, 78). Production of superoxide anion is increased in IR owing to activation of NADPH oxidase, resulting in accumulation of ROS. In turn, oxidative stress can reduce insulin sensitivity, thereby worsening existing IR (79).

Scavenger Receptors

CD36 is a scavenger receptor for modified LDL, and plays an important role in foam cell formation. CD36 is a marker of macrophage activation (80), and its expression on monocytes is upregulated by ox-LDL (81). The importance of CD36 in CVD has been emphasized by the demonstration that CD36 knockout mice have a reduction in atherosclerotic lesion formation (81), probably through inhibition of foam cell formation. High glucose levels, both in vivo and in vitro, are associated with upregulation of CD36 expression on monocytes (82, 83), and plasma-soluble CD36 levels are elevated in obese subjects and in those with diabetes (84). However, it remains unclear whether upregulation of CD36 production in an IR state is directly implicated in the development of atherosclerosis or whether it simply represents an epiphenomenon, secondary to a generalized inflammatory state.

Systemic inflammatory changes in CVD

In addition to local inflammation, the atherosclerotic process is associated with a generalized inflammatory reaction. Plasma levels of inflammatory molecules are increased in patients with coronary artery disease, including IL-1, IL-6, C-reactive protein (CRP), and complement C3 (85, 86). CRP has received major interest, as it is present in atherosclerotic plaques early in the disease process and associates with foam cells to suggest a direct role for this protein in atheroma formation (87, 88). Plasma levels of CRP predict future thrombotic events and may therefore be of clinical prognostic value (89). The complement system has also been under the spotlight, as complement C3 plasma levels predict risk of MI and complement components are also detected in atherosclerotic plaques (90, 91). The failure of atherosclerotic progression beyond the foam cell stage in C3-deficient mice suggests a direct role for this protein in atheroma formation (92). Interestingly, recent work suggests that C3 may be a better indicator of the atherosclerotic burden than CRP, emphasizing the importance of this molecule in the atherosclerotic process (86). The inflammatory reaction and early vascular changes can be detected in obese children with IR (93), indicating that the pathogenic process can start at a very young age, and therefore effective strategies to reduce weight in obese children should be developed in order to reduce future cardiovascular risk.

Insulin resistance and inflammation

The association between inflammation and diabetes was documented more than a century ago in 1876, when Ebstein demonstrated that high-dose salicylate can improve the symptoms of diabetes (94). However, it was only recently that a link between IR and inflammation was suggested by the demonstration that amelioration of IR occurred in mice treated with the soluble TNFα receptor (95). Further

work has shown that plasma concentrations of TNFα are elevated in obese individuals, and ameliorated by weight loss (96, 97). Plasma levels of other inflammatory molecules, including IL-6, IL-18, CRP, and C3, are also elevated in IR states and in diabetes, confirming an association between impaired glucose metabolism and a generalized inflammatory state (98–102). Some of these inflammatory molecules, such as IL-8 and IL-18, are produced mainly by non-adipose tissue; others, such as C3, TNFα, and IL-6 are produced both by adipose and non-adipose tissue, whereas some are mostly adipose tissue products, such as leptin and adoponectin (103), directly implicating adipocytes in the inflammatory state associated with atherothrombotic disease. The ability of preadipocytes to acquire macrophage-like properties during cell maturation stresses the importance of the contribution of adipose tissue to the inflammatory state seen in obesity (104).

Weight loss is associated with a reduction in markers of generalized inflammation (105–107), supporting the concept of a direct involvement of adipose tissue in the inflammatory reaction seen in IR. However, the amelioration in the inflammatory response by the insulin sensitizers thiazolidinediones (TZDs) is not associated with weight loss (108), suggesting that the association between adiposity and inflammation is complex and is not simply a matter of "adipose tissue quantity" but also involves qualitative changes in adipocyte function.

A number of mechanisms seem to be responsible for the association between IR and inflammation. Chronic inflammatory states induce IR through interference with the insulin signaling pathway (103) as well as by inducing alterations in adipocyte properties (109). IR, in turn, predisposes to inflammation through increased oxidative stress that acts locally on the vessel wall triggering an inflammatory reaction, and generally on adipose tissue, stimulating adipocytes to produce inflammatory cytokines (103).

The ability of fat cells to produce molecules involved in inflammation as well as glucose and lipid metabolism may explain the close association among obesity, IR, and inflammation (110, 111). Of these, leptin and adiponectin, both of which are adipocyte products, have received major interest. Animal studies have shown that adiponectin overexpression results in amelioration of IR (112). This observation, together with the finding of increased adiponectin levels after treatment with insulin sensitizers (113), directly implicates this protein in amelioration of IR. At the vascular level, adiponectin protects from atherothrombosis through a number of mechanisms, including the downregulation of adhesion molecule expression and inhibition of VSMC proliferation (114). On the other hand, the role of leptin in IR and inflammation seems to be more complex. Subjects with lipodystrophy and leptin deficiency have severe IR that can be improved by leptin administration (115). However, in obese individuals with IR, leptin levels are increased, suggesting an association between leptin resistance and IR (116). Leptin has atherothrombotic properties, as it augments oxidative stress and the inflammatory response in the vessel wall (117), and may initiate the thrombotic process by activating platelets (118). Furthermore, leptin induces a generalized inflammatory response by stimulating adipose tissue IL-6 production (119). Therefore, leptin promotes atherothrombosis, while adiponectin protects against both IR and vascular damage.

The clinical importance of these hormones has been suggested by the demonstration of a positive correlation between leptin levels and risk of MI, and a negative correlation between adiponectin levels and MI risk (120, 121).

In summary, IR states, which are usually associated with obesity, increased oxidative stress, and generalized inflammation, play a crucial role in the development and progression of atherosclerosis. The generalized inflammatory state further augments IR by interfering with insulin signaling and adipocyte function, creating in the process a vicious cycle that maintains an environment favoring the development of vascular damage.

Thrombosis

Blood clot formation

Plaque rupture results in the exposure of tissue factor (TF) bearing cells and the adhesion of partially activated platelets to the site of injury, initiating occlusive clot formation. TF binds FVII and this complex activates FIX, FX, and FV (122, 123), resulting in thrombin generation, which fully activates adherent platelets and maintains thrombin generation (Fig. 11.1). Thrombin also converts fibrinogen to fibrin to initiate fibrin clot formation and activates FXIII to crosslink fibrin fibers resulting in stabilization of the clot. A defense mechanism against thrombosis occurs via natural anticoagulants, including protein C, which inhibits factors V and VIII; tissue factor pathway inhibitor, which blocks TF-FVII initiation of coagulation; and thrombomodulin, which inhibits thrombin activity (124, 125). Once a blood clot is formed, plasmin is generated on the clot surface from plasminogen by the action of tissue plasminogen activator (tPA), and plasmin breaks down fibrin to release fibrin degradation products (FDPs; Fig. 11.1) (126). A number of proteins control and inhibit the fibrinolysis reaction including plasmin inhibitor (formerly α2-antiplasmin), which binds and inhibits plasmin, and plasminogen activator inhibitors (PAI), which interfere with plasminogen activation by binding to, and inhibiting, tPA. (127).

Clotting factor, clot structure, and platelets

Atherosclerotic disease is associated with both increased levels of clotting factors and inhibition of fibrinolysis. Plasma levels of TF and fibrinogen are elevated in patients with coronary artery disease, and disease severity seems to correlate with levels of these proteins (128–131). In addition, increased PAI-1 levels occur in patients with atherosclerosis, resulting in inhibition of tPA leading to hypofibrinolysis (132–135).

Other than quantitative changes in clotting factors, the final clot structure has an important role in determining risk of atherothrombotic disease. Studies ex vivo

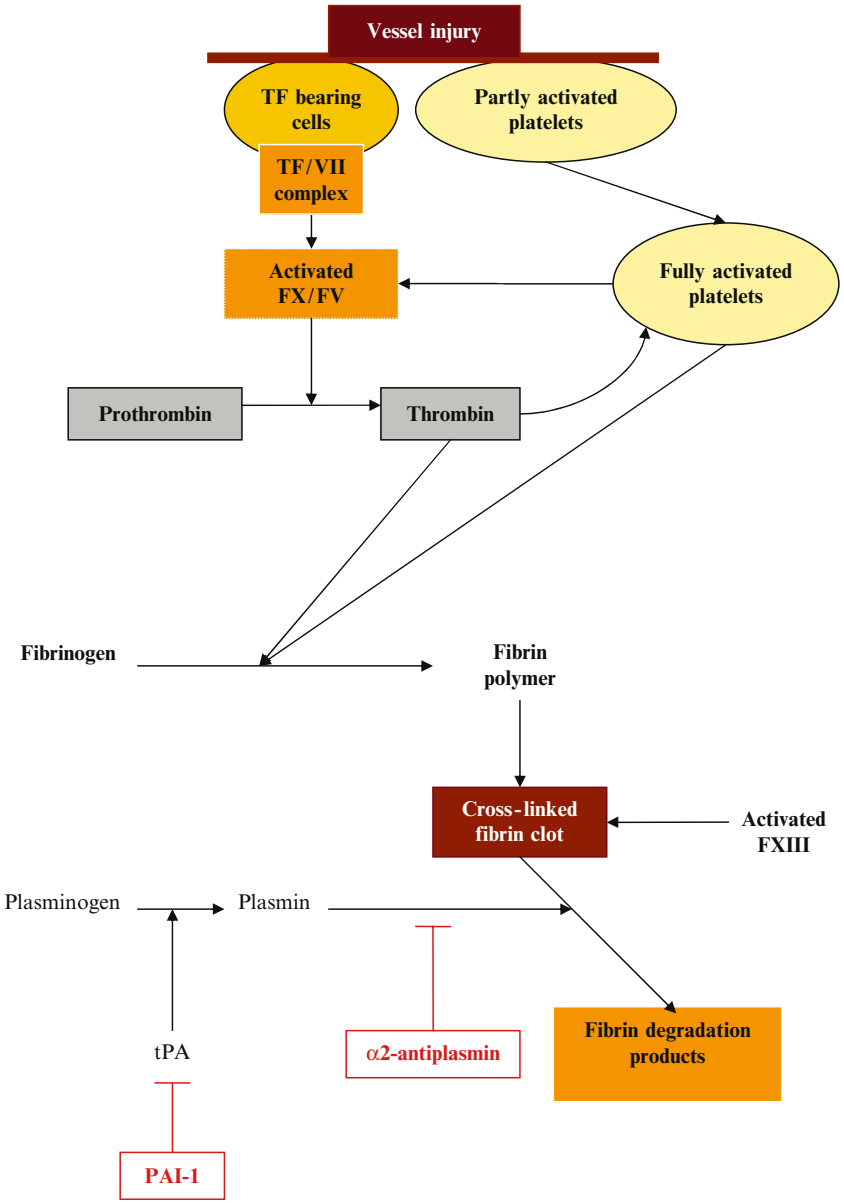

Fig. 11.1 The coagulation cascade in atherothrombotic disease. A break in the vessel wall brings the plasma into contact with tissue factor (TF) bearing cells, and platelets adhere to the site of injury and become partially activated. FVII/TF complex activates other clotting factors, which subsequently cleave prothrombin to generate thrombin, which fully activates the attached platelets, and this helps to generate enough thrombin to maintain clot formation, consequently resulting in the formation of a stable hemostatic plug.Fibrin clot lysis is initiated by tissue plasminogen activator (tPA) that mediates the activation of plasminogen to plasmin. Inhibitors of fibirnolysis include plasminogen activator inhibitor (PAI-1), which inhibits tPA, and α2-antiplasmin, which inactivates plasmin (*See* Color Plate 3)

have shown that clots composed of thin fibers and small pores are associated with an increased risk of CVD (136), which may be related to slower rates of clot lysis associated with this structure. Patients with T2DM tend to generate a tight clot structure with associated impairment of fibrinolysis. These changes in clot structure are more marked in subjects with poor glycemic control, suggesting a mechanism for the increased thrombotic risk in diabetic subjects (137). Genetic variations in clotting factors may also have a role in determining cardiovascular risk through an effect on clot structure. For example, a common polymorphism in the β-chain of fibrinogen resulting in Arg to Lys amino acid substitution at position 448 has been shown to be associated with more severe coronary artery disease (138), which may be related to alteration in clot structure (139), emphasizing the importance of genetic factors in predisposition to CVD, and perhaps explaining some aspects of familial predisposition to disease.

Platelet reactivity is associated with increased atherothrombotic events (140, 141), and an association between platelet size (a marker of platelet activation) and CVD has been shown (142).

Insulin resistance and thrombosis

Alterations in coagulation factors have been documented in diabetic and IR states. TF activity is increased in diabetes and tends to normalize by improving glycemic control (143). A number of coagulation factors are increased in relation to features of IR (fibrinogen, Factor VII and Factor XII), and there is some evidence that activation of some clotting factors is enhanced in the presence of triglyceride-rich particles. Diabetes itself is associated with an increase in fibrinogen levels (144), which correlates with the degree of hyperglycemia, although the effect of improved glycemic control on fibrinogen levels is unclear. PAI-1 levels are closely correlated with IR and associated risk factors including blood pressure and plasma lipids (145–147). Hyperglycemia is associated with glycation of fibrinogen, the extent of which correlates well with glycemic control (148). Clots formed from diabetic subjects are tighter and less porous, with altered tPA, plasminogen, and plasmin inhibitor binding to fibrin, resulting in decreased plasmin generation and slower clot lysis rates (135, 147, 148). These findings suggest that glycation of fibrinogen may have a role in increased cardiovascular risk (149). Glucose levels also appear to modulate clot structure, as both hypo- and hyperglycemia induce a fibrinolytic-resistant fibrin structure.

Impaired insulin sensitivity leads to the loss of platelet-inhibitory activity in insulin-resistant individuals (150–152). Furthermore, obesity is associated with increased platelet activation, which can be ameliorated by weight loss, thereby directly implicating IR in enhanced platelet activation (153, 154). In addition, endothelial dysfunction, secondary to IR, leads to reduced NO bioavailability and enhanced platelet aggregation. A complete understanding of the mechanisms leading to increased platelet activation in IR is not available, but may involve decreased

endothelial NO production, oxidative stress, increased platelet turnover, altered platelet structure due to dyslipidemia, and disproportionate increase in intra-platelet calcium concentration (155–159).

Endothelial dysfunction, inflammation, and thrombosis

There is close association among endothelial dysfunction, inflammation, and thrombosis. EC have the capability to produce a large number of inflammatory and adhesion molecules contributing to local and generalized inflammation (160). EC also modulate thrombosis potential by secreting NO, a powerful antiplatelet agent, prothrombotic molecules such as TF and PAI-1, and anti-thrombotic agents including protein C, protein S, and thrombomodulin (161, 162). In turn, inflammatory molecules and oxidative stress can both aggravate endothelial dysfunction and may play a role in weakening the fibrous cap, resulting in plaque rupture and initiation of thrombosis (163). Thrombus formation further enhances endothelial dysfunction by inducing ischemic changes in EC, thereby accelerating the atherosclerotic process.

An interaction between inflammation and thrombosis is also evident, as raised CRP is associated with increased fibrinogen levels together with reduced fibrinolytic activity (164). Cytokines induce TF expression on EC, inhibit NO secretion, and limit protein C activation and thrombomodulin production, creating a prothrombotic and vasoconstrictive environment (165–167). The interaction between inflammation and coagulation is not limited to the site of vessel injury, as IL-6 stimulates hepatocytes to produce fibrinogen and PAI-1, thereby inducing a generalized prothrombotic state (168). On the other hand, platelets and coagulation proteins may modulate the inflammatory response, as activated platelets increase inflammation through the release of chemotactic factors (169), whereas protein C and thrombomodulin have antiinflammatory activity (170, 171), and downregulation of these molecules may result in intensification of the inflammatory response. Therefore, it appears that inflammation is associated with upregulation of procoagulant factors and inhibition of fibrinolytic activity, whereas loss of anticoagulant activity and stimulation of the coagulation system are in turn associated with an increased inflammatory response.

The role of IR in the development of atherothrombotic disease is summarized in Fig. 11.2.

Insulin resistance and traditional risk factors

Although endothelial dysfunction, inflammation, and enhanced coagulation are central to the pathogenesis of atherothrombotic disease, they are not routinely assessed in clinical practice. Direct assessment of endothelial function is a complex

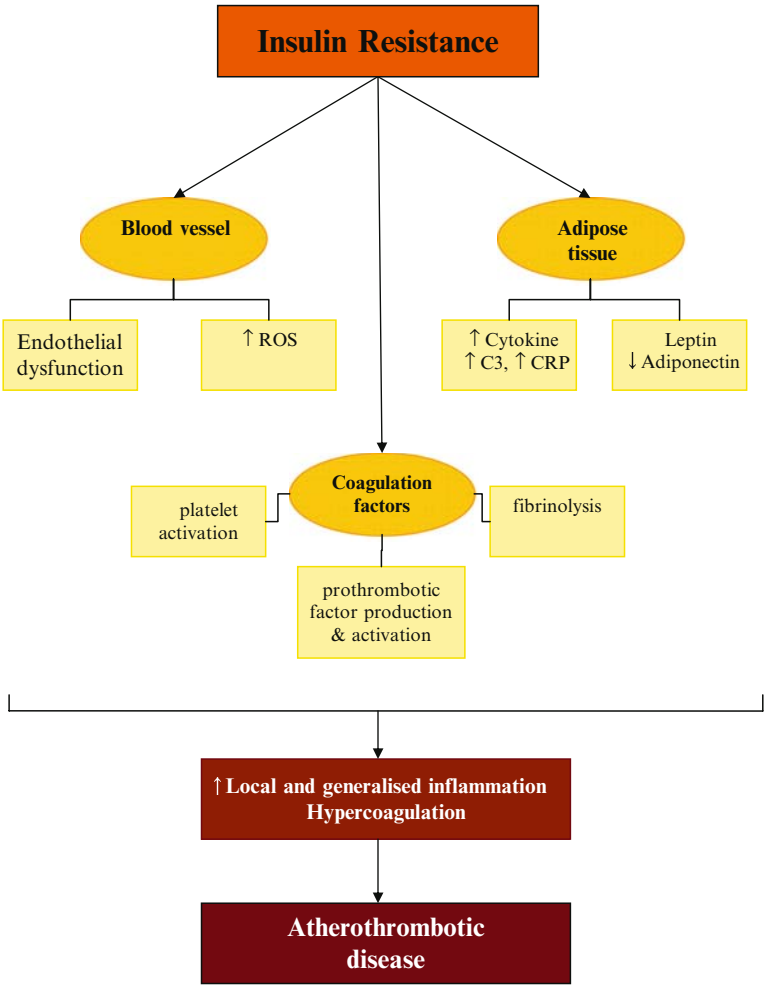

Fig. 11.2 The role of insulin resistance in the atherothrombotic process. Insulin resistance is associated with endothelial dysfunction and the accumulation of reactive oxygen species (ROS) in the vessel wall that help to start and maintain the atherosclerotic process. Insulin resistance affects the adipocyte function, resulting in the generation of a number of inflammatory molecules, which play a key role in the atherosclerotic process. Furthermore, insulin resistance is associated with increased platelet activation as well as increased level and activity of prothrombotic factors, thereby ensuring the presence of a prothrombotic milieu. CRP: C-reactive protein; C3: complement C3 (*See* Color Plate 4)

process and relies on studying flow-mediated dilatation in an artery, a technique that remains confined to research work at present. Equally, measurement of plasma levels of clotting factors and analysis of clot structure are expensive and labor-intensive, and are currently used only for research purposes. On the other hand,

measurement of inflammatory markers, particularly CRP, is relatively easy and is used in some centers to help with risk stratification.

There is a close association between IR and hypertension that is evident even in children, and a number of mechanisms have been implicated, including endothelial dysfunction (detailed above), increased sympathetic activity, and altered activity of the renin–angiotensin system (14, 172). IR is also associated with deranged lipid metabolism, in both young and older subjects, manifested as raised triglycerides and low HDL (173, 174), resulting in an atherogenic lipid profile.

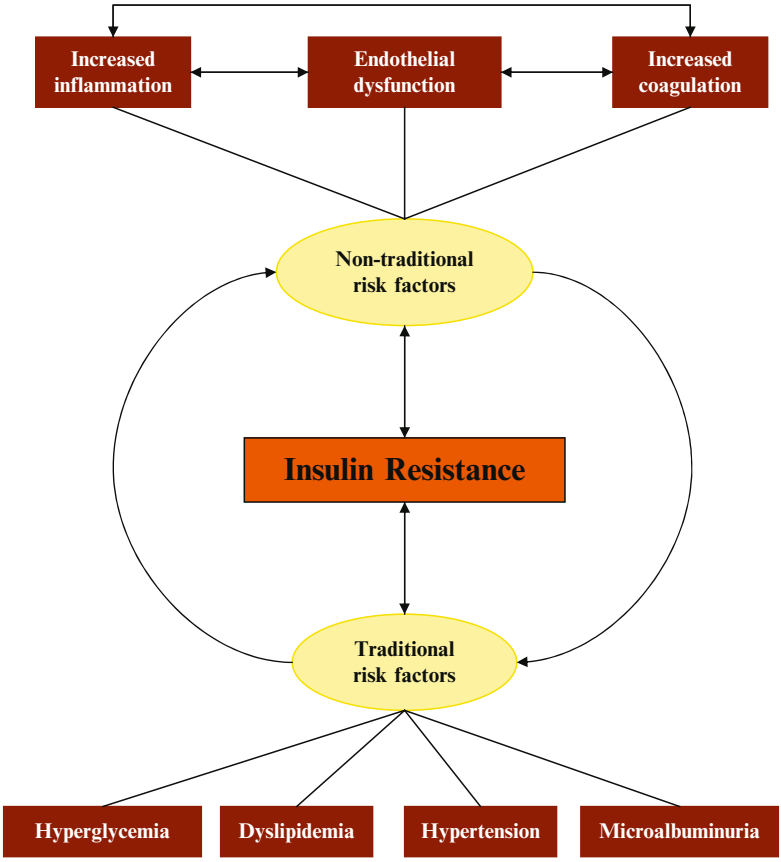

Fig. 11.3 The relationship between insulin resistance and traditional/non-traditional risk factors for atherothrombotic disease. Insulin resistance is associated with traditional risk factors for atherothrombotic disease including hyperglycemia, dyslipidemia, hypertension, and microalbuminuria. Insulin resistance is also associated with non-traditional cardiovascular risk factors including endothelial dysfunction, increased inflammation, and hypercoagulation. Furthermore, there is an interaction between traditional and non-traditional risk factors, and both can worsen insulin resistance, consequently leading to a vicious cycle that promotes the development of atherothrombotic disease (*See* Color Plate 5)

Microalbuminuria is an important risk factor for cardiovascular mortality and is clearly associated with IR (175–177) through a number of mechanisms including endothelial dysfunction, increased renal capillary permeability, glomerular hyperfiltration, and increased glomerular capillary pressure (178–180). Therefore, microalbuminuria is not only an indicator of early renal damage but also a marker of endothelial dysfunction and a generalized increase in vascular permeability.

The interaction between traditional and non-traditional risk factors in relation to IR is summarized in Fig. 11.3.

Improving Insulin Sensitivity and Its Role in the Prevention of Diabetes and Cardiovascular Disease

It is well established that introducing measures to improve IR both prevents the development of T2DM and has beneficial effects on cardiovascular risk, including glucose control, blood pressure, microalbuminuria, and lipid profile (181). In the Diabetes Prevention Program (DPP), treatment with metformin resulted in a 31% reduction in the development of diabetes in individuals with impaired glucose tolerance over a period of 2.8 years (182). The troglitazone in the Prevention Of Diabetes mellitus trial (TRIPOD) showed that the insulin sensitizer troglitazone reduced the risk of developing diabetes by 56% over 30 months in Hispanic women with a previous history of gestational diabetes (183). A follow-up study in the same cohort of patients has shown that pioglitazone, a TZD in current use, further prevents the development of diabetes (184). The recently reported Diabetes REduction Assessment with ramipril and rosiglitazone Medication (DREAM) study has shown that rosiglitazone reduces the development of diabetes in subjects with impaired fasting glucose or impaired glucose tolerance (IGT) over a period of 3 years by ~62% (185).

Other than insulin sensitizers, a recent study has shown that treatment of subjects with a body mass index (BMI) over 30 with the weight-reducing agent orlistat resulted in 37% reduction in the conversion to diabetes over a 4-year period, which was associated with improvement in insulin sensitivity, emphasizing the close association between obesity and abnormal glucose metabolism (186).

These studies, however, were not adequately powered to demonstrate a protective effect from CVD, and it remains unclear whether medical intervention with insulin sensitizers in subjects with IGT has an additional cardioprotective effect.

However, in contrast to our current knowledge with regard to IGT, the use of insulin sensitizers in patients with established diabetes has been shown to protect from CVD. In the United Kingdom Prospective Diabetes Study (UKPDS), metformin use in overweight individuals was associated with a significant decrease in cardiovascular risk (187), this being one of the main reasons why metformin is used as first-line treatment in overweight T2DM. More recently, the PRO-active study has reported that pioglitazone had significant effects on the secondary end points of the trial (stroke, MI, cardiovascular death) in high-risk patients with diabetes and

established CVD over a follow-up period of 3.4 years (190). Unfortunately, in this study the primary end point, which included revascularization, was nonsignificant, a finding that has led to protracted debate about the meaning of these results. Subsequent subgroup analyses of acute coronary syndromes and thrombotic stroke, in which inflammatory thrombotic risk predominates, have, however, been reported as strongly positive in favor of pioglitazone, supporting the view that the drug is cardioprotective. An additional finding of the PROactive study was that TZD treatment was associated with a 50% reduction in progression to permanent insulin use due to an improvement in insulin sensitivity. This latter observation has important ramifications with respect to the clinical use of TZD in this population.

The mechanism by which metformin and TZDs offer cardiovascular protection is probably multifactorial. Metformin has a modest beneficial effect on lipid profile, and its use is associated with a reduction in both oxidative stress and plasma CRP levels (189–191). Metformin affects the coagulation system by inhibiting platelet aggregation and reducing levels of fibrinogen and PAI-1, and it also has a beneficial effect on clot structure (192, 193). The effects of metformin are probably secondary to improvement in IR and restoration of endothelial function (194).

TZDs have an even more extensive list of effects including favorable effects on lipid profile, blood pressure, microalbuminuria, and inflammation. (181). TZDs reduce PAI-1 levels, to improve fibrinolysis and decrease MMP-9 levels, indicating a role in maintaining plaque stability (181). TZDs also reduce platelet activity in non-diabetic subjects who have coronary artery disease (195), which may be an indirect effect through improvements in endothelial function and restoration of NO production.

The association between childhood cardiac risk factors and vascular disease in later life (18, 196), together with autopsy studies demonstrating arterial abnormalities in the first two decades of life in high risk subjects (197), indicates that intervention at an early age is necessary to prevent future CVD. As IR plays a central role in the atherothrombotic process, introducing measures to improve insulin sensitivity in children and young adults will have a key protective role.

The role of insulin sensitizers in the prevention of diabetes and CVD is summarized in Table 11.1.

Table 11.1 Role of improving insulin sensitivity in the prevention of diabetes and cardiovascular disease (CVD)

Agent	Prevention from diabetes	Prevention from CVD
Metformin	Yes (184)	Yes (189)
Rosiglitazone	Yes (187)	Unclear
Pioglitazone	Yes (186)	Yes (190)
Orlistat	Yes (188)	Unknown

Conclusion

The close association among obesity, diabetes, and atherothrombotic disease is due to clustering of risk factors, arbitrarily divided into traditional (hyperglycemia, dyslipidemia, hypertension, and microalbuminuria) and non-traditional (endothelial dysfunction, increased inflammation, and hypercoagulation). These risk factors commonly associate with IR, which is involved in the development of atherothrombosis and which is detected at a very young age in the presence of childhood obesity. The decreased tissue sensitivity to insulin results in excessive insulin production by the pancreas, which eventually fails to keep up with the increased demand, leading to secondary β-cell failure and the development of diabetes. IR predisposes to atherothrombosis through a number of mechanisms including dysglycemia, oxidative stress, endothelial dysfunction, modulation of adipocyte function, and level/activity of coagulation factors, creating an inflammatory and prothrombotic environment.

Although medical intervention to prevent/treat atherothrombotic disease can be effective to some extent, it is far from ideal and novel treatment strategies are needed. The association between IR and endothelial dysfunction, inflammation, and increased thrombosis indicates that impaired insulin sensitivity plays a role in disease pathogenesis both early and late in the atherothrombotic process. Direct, early modulation of insulin sensitivity offers a unique way of prevention from and treatment of diabetes and CVD.

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Chapter 12
Insulin Resistance and Cardiovascular Disease

Cecilia C. Low Wang

Keywords atherosclerosis, insulin resistance, type 2 diabetes mellitus, cardiovascular disease, metabolic syndrome X, visceral adiposity, compensatory hyperinsulinemia, inflammation, oxidative stress, endothelium, vascular smooth muscle, intracellular signaling proteins, early growth response 1 protein, primary prevention, lifestyle risk reduction

Introduction

Atherosclerosis is a major cause of debilitation and mortality in the United States and worldwide. Its sequelae of heart disease and strokes account for approximately one-third of all deaths in the United States (1). Individuals with metabolic syndrome and/or type 2 diabetes (T2DM) are at markedly increased risk for developing atherosclerosis. Furthermore, cardiovascular disease (CVD) is the leading cause of death in diabetes (2). Unfortunately, a recent analysis of data from the Framingham Heart Study shows that the proportion of CVD that can be attributed to diabetes has increased over the 50 years of the study (3). Insulin resistance is a key underlying feature of both metabolic syndrome and T2DM. Specific mechanisms for the association between insulin resistance and atherosclerosis are incompletely understood, but many investigators have shed light on this complex and important clinical problem.

Background

The link between coronary heart disease and diabetes was noted in 1883 by Vergely (4). The increased incidence of CVD in diabetes has been corroborated in many subsequent studies (5–7). Furthermore, investigators have demonstrated that plasma insulin levels in the highest quintile of the populations studied predict the development of CVD in subjects without diabetes (8–11). On the other hand, "android obesity", or what is now termed "visceral adiposity", was noted to be strongly associated with diabetes and atherosclerosis over half a century ago (12) and

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confirmed by many investigators since then (13, 14). The term "Syndrome X" was coined by Reaven in a Banting lecture (15) to encompass several metabolic abnormalities noted to occur in the same individual and postulated to be important for the pathogenesis of coronary artery disease. As described by Reaven initially, this syndrome included resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased very low density lipoprotein (LDL) triglyceride, decreased high density lipoprotein (HDL) cholesterol, and hypertension. This syndrome has been termed the "insulin resistance syndrome" (16), and most recently, "metabolic syndrome" (17). Additional factors have been added to this cluster including visceral adiposity, endothelial dysfunction, inflammation, small dense LDL cholesterol, pro-coagulant factors, and hyperuricemia.

Insulin resistance plays a central role in the pathophysiology of T2DM and metabolic syndrome (15, 18). Insulin resistance is defined clinically as a situation in which higher than usual concentrations of insulin are needed to maintain normoglycemia (19). There are several hallmarks of insulin resistance, including selective impairment of the phosphatidylinositol (PI) 3-kinase intracellular signaling pathway in response to insulin, compensatory hyperinsulinemia, intact signaling along the extracellular signal-regulated kinase (ERK) 1/2 mitogen-activated protein (MAP) kinase signaling pathway, elevated free fatty acids, and inflammation (20–25).

Compensatory hyperinsulinemia arises to promote glucose uptake in adipose tissue and skeletal muscle, and glucose utilization in all insulin target tissues (20, 21). Results have been mixed in proving whether compensatory hyperinsulinemia in the setting of insulin resistance has any independent detrimental effects or whether it is merely a marker of insulin resistance, with insulin resistance exerting its pro-atherogenic action via other mechanisms. Several retrospective clinical studies support the hypothesis that hyperinsulinemia *per se* is detrimental, increasing the risk for CVD and other diseases (11, 26), while others do not support the idea that hyperinsulinemia is an independent cardiovascular risk factor (27, 28).

On the other hand, numerous studies demonstrate associations among insulin resistance, CVD, and mortality (29–32). Furthermore, insulin resistance itself, as determined by decreased insulin-mediated glucose disposal, predicts that increased CVD risk (33), insulin sensitivity (determined by the frequently sampled intravenous glucose tolerance test and the Bergman minimal model), and atherosclerosis, measured by carotid artery intimal-medial thickness (IMT), are directly related (34).

Resistance to insulin action at the level of skeletal muscle results in impairment of glucose disposal, whereas insulin resistance at the level of adipose tissue results in loss of inhibition of lipolysis, resulting in high circulating free fatty acids that provide ample substrate for excessive hepatic triglyceride production. At the same time, insulin resistance at the level of the liver results in excessive hepatic glucose output that contributes to compensatory hyperinsulinemia. Lastly, visceral adiposity results in secretion of inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and relatively low adiponectin levels. Determining the precise mechanisms for insulin-resistance-associated atherosclerosis and CVD has been difficult because of the abnormalities often associated with insulin resistance: abnormal lipid metabolism with elevated triglycerides, low HDL cholesterol and

increased small, dense LDL, hypertension, visceral adiposity, inflammatory changes in the vascular wall, endothelial dysfunction, and pro-coagulant factors. It is unclear which of these are the most important in initiation and progression of atherosclerosis, but it is likely that they all play key roles (Fig. 12.1).

Importantly, individuals with insulin resistance are at high risk for developing not only coronary artery disease, but also congestive heart failure (35). Conversely, individuals with congestive heart failure are at higher risk for developing insulin resistance and diabetes (36). Positron emission tomography studies show decreased myocardial and skeletal muscle glucose uptake in insulin-resistant subjects with known coronary artery disease compared with controls (37, 38). These studies have been supported by invasive coronary sinus and forearm studies, showing decreased myocardial and forearm glucose uptake in subjects with angina, abnormal exercise treadmill testing, and normal coronary angiography (39). Since then, many investigators have helped to elucidate mechanisms for cardiac dysfunction in diabetes and insulin resistance, but this will not be a focus of the present review.

Atherosclerosis

Atherosclerosis was initially thought to start with endothelial denudation in response to injury, with progressive accumulation of lipids and growth factor stimulation of smooth muscle cell proliferation resulting in an inert atheromatous lesion

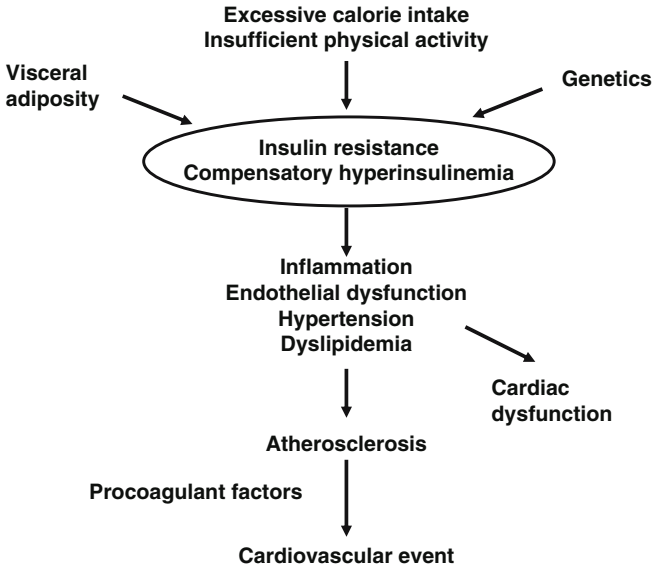

Fig 12.1 Contribution of insulin resistance and hyperinsulinemia to development of atherosclerosis and cardiovascular risk

of lipid debris surrounded by smooth muscle cells (40, 41). This hypothesis was later modified to identify endothelial dysfunction rather than denudation as the initial key element. The concepts of atherogenesis and atherosclerosis progression have since further evolved with the accumulation of strong evidence from bench and clinical research demonstrating central roles for inflammation and oxidative stress. Atherosclerosis is now understood to consist of several stages involving multiple interrelated processes including endothelial dysfunction, lipid abnormalities, inflammation, oxidative stress, vascular smooth muscle cell (VSMC) activation, arterial wall remodeling, platelet activation and thrombosis, and genetic factors (41). Atherosclerosis is now known to also involve the immune system, with inflammation playing a key role (42, 43).

In response to an atherogenic diet, circulating monocytes and T lymphocytes attach to endothelial cells that line the arterial intima. This often occurs in areas of turbulent blood flow instead of laminar shear stress, and can result from modified lipoprotein and pro-inflammatory cytokine induction of vascular cell adhesion molecule-1, P- and E-selectins, and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells via nuclear factor-kappa beta (NF-κB). Once leukocytes adhere to the arterial intima, they migrate through the endothelium in response to monocyte chemoattractant protein-1 (MCP-1), IL-1 and -8, and interferon-γ. Activated leukocytes release enzymes, growth factors, and cytokines including matrix metalloproteinases that degrade matrix proteins and further induce endothelial cells, smooth muscle cells, and leukocytes themselves in a feed-forward fashion. In the arterial intima, monocytes become more macrophage-like in response to macrophage colony stimulating factor and express scavenger receptor A, CD36, and CD40. These macrophages internalize modified lipoproteins and become foam cells. All these cellular components form the initial atheromatous lesion. VSMC migration, proliferation, and apoptosis of cells within the plaque result in the growth of the atherosclerotic plaque.

However, instead of uninterrupted progression of the atheroma with resultant luminal narrowing and occlusion of coronary vessels, we now understand that most acute coronary events occur because of thrombosis, hemorrhage, and, possibly, fibrous cap disruption of non-stenotic atherosclerotic plaques, precipitating myocardial ischemia and infarction. The inflammatory cytokines, adhesion molecules, chemoattractant molecules, proteolytic enzymes, and growth factors involved in atherogenesis and atheroma progression are produced by endothelial cells, VSMCs, T lymphocytes and macrophages. Whether the endothelial cells and smooth muscle cells are resident cells or whether they originate from circulating stem cell precursors is an area under active investigation (44–47).

Oxidative stress, which is increased in diabetes, is implicated in the pathogenesis of atherosclerosis (48–50). In biological systems, oxygen is reduced to form superoxide anion via nicotinamide adenine dinucleotide (phosphate) (NADH/NAD(P)H) oxidases and xanthine oxidases, or through reaction with compounds in the mitochondrial electron transport chain. Superoxide anion is then converted to hydrogen peroxide either nonenzymatically or through the action of superoxide dismutases (SOD). Hydrogen peroxide can react further to produce hydroxyl radicals. Cells

have developed many mechanisms for protection against excessive oxidant stress including SOD, catalase, and glutathione peroxidase, and the use of antioxidants such as glutathione and ascorbate. Unfortunately, in situations such as atherosclerosis and diabetes, excessive production of reactive oxygen species (ROS) by the cellular components of atheromas can overwhelm cellular oxidant scavenging capacity, resulting in oxidative stress and subsequent damage to DNA, lipids, and proteins, impairment of gene transcription, and increased production of pro-inflammatory transcription factors. The enhanced production of superoxide anion observed in atherosclerosis can also result in increased reactivity with nitric oxide (NO), with resulting production of an extremely toxic oxidant, peroxynitrite. Downregulation of SOD by turbulent shear stress may also create a pro-atherogenic environment. Angiotensin II also plays a key role in modulating the activation of endothelium and smooth muscle cells and may induce oxidative stress by upregulating the NF-κB pathway. In addition, there is evidence for increased mitochondrial production of ROS as a common mechanism for several pathways leading to microvascular complications of diabetes (51), and accumulating evidence for this common mechanism in macrovascular disease associated with insulin resistance. Finally, free fatty acid flux may also play a key role (52), and has been shown to increase ROS by activating pathways also affected by hyperglycemia: advanced glycation end products, protein kinase C, the hexosamine pathway, and NF-κB (53, 54).

Insulin resistance and vascular disease

The importance of the endothelium for normal vascular function and the pathogenesis of atherosclerosis began with the discovery that endothelial cells were necessary for acetylcholine-mediated vasodilation (55). The endothelium produces many mediators, including NO, endothelin, and angiotensin II, that balance the functions of vasodilation and vasoconstriction, hemostasis, angiogenesis, and vascular permeability (56). Initially, endothelial dysfunction was defined as impaired vasodilation in response to specific substances such as acetylcholine or bradykinin. This definition has since been broadened to include the pro-inflammatory and prothrombotic state associated with damage and dysfunction of the endothelium. In healthy subjects, activation of endothelial NO synthase (eNOS) results in arterial vasodilation, whereas a similar stimulus results in paradoxic vasoconstriction of the coronary arteries in subjects with coronary artery disease (57). Furthermore, endothelial dysfunction can be demonstrated in subjects who smoke but have no detectable atherosclerosis (58) and in subjects who are insulin resistant (59), suggesting that endothelial dysfunction is a key element of atherosclerosis in the setting of known risk factors for CVD.

Endothelial-derived NO correlates with the degree of insulin sensitivity and may be a key link between insulin resistance and endothelial dysfunction (60). Insulin increases the expression and activity of eNOS in cultured endothelial cells and microvessels from lean, insulin-sensitive Zucker rats, but not in fat, insulin-resistant

Zucker rats (61). This suggests that insulin regulates NO production constitutively, but that this regulation is impaired in insulin resistance leading to endothelial dysfunction.

Insulin resistance is often associated with obesity. Numerous studies in recent years have demonstrated that adipose tissue is metabolically active. It acts in both an endocrine and paracrine fashion to produce inflammatory mediators such as plasminogen activator inhibitor-1, TNF-α, and IL-6, and hormones or adipokines including leptin, adiponectin, resistin, and retinol-binding protein-4 (RBP-4) (62–64). Inflammatory stimuli activate several pathways including the NF-κB pathway. Activation of NF-κB occurs upon degradation of inhibitor of κB, which releases NF-κB, allowing it to translocate to the nucleus and induce transcription of pro-inflammatory cytokines in a feed-forward loop. These factors are now thought to play much more important roles in obesity-associated insulin resistance and cardiovascular risk than non-esterified fatty acids alone. Visceral adiposity in particular is correlated with cardiovascular events and mortality (65, 66).

Peroxisome proliferators-activated receptors (PPARs) are nuclear receptors that heterodimerize with retinoic X receptors to regulate carbohydrate and lipid metabolism. Thiazolidinediones (TZDs) are a class of drugs known to target PPAR-γ and used to treat diabetes and insulin resistance, while fibric acid derivatives used to treat hypertriglyceridemia target PPAR-α. The potential role of PPARs in insulin-resistance-associated atherosclerosis and CVD is multifaceted. Rodents with adipose-skeletal-muscle- or liver-specific deletions of PPAR-γ are more likely to develop insulin resistance (67–69). Treatment with PPAR-γ ligands inhibits VSMC expression of matrix metalloproteinase-9, reduces platelet-derived growth factor-induced VSMC migration (70), and inhibits VSMC proliferation (71). Transplantation studies have demonstrated that PPAR-γ in the macrophage helps protect against the development of atherosclerosis (72), while TZD therapy in rodent models reduces atherosclerosis (73).

TZD therapy may improve endothelial dysfunction in individuals with multiple cardiovascular risk factors (74), but studies of direct TZD effects on atherosclerosis in humans have had mixed results (75–77). PPAR-α activation improves endothelial function, possibly through its effect on lipid parameters, markers of inflammation, and adiponectin levels (78,79). Activation of PPAR-α also improves insulin sensitivity in humans (80). Importantly, deficiency of PPAR-α in a rodent model exhibited smaller atherosclerotic lesion area and lower blood pressure, despite increased VLDL production (81). A potential role for PPAR-δ in atherosclerosis was demonstrated by transplantation of PPAR-δ-deficient bone marrow into an atherosclerosis-prone mouse model (82). Recipients developed much smaller atherosclerotic lesions and decreased inflammatory mediators. However, the utility of PPAR-δ ligands for atherosclerosis is still unclear, and studies have been equivocal (83).

Another possible link between insulin resistance and atherosclerosis is the renin–angiotensin–aldosterone system. Hypertension is a common condition in individuals with insulin resistance. Angiotensin II induces insulin resistance and impairs insulin signaling via increased serine phosphorylation of the insulin receptor and insulin receptor substrate-1 (84,85). Early studies also showed enhanced

responsiveness to angiotensin II in hypertensive individuals, which correlated with insulin sensitivity (86). Angiotensin II induces VSMC proliferation and migration and reduces VSMC responsiveness to NO (87–89). Inhibition of the renin–angiotensin system decreases inflammation, including MCP-1 expression and NF-κB activation, decreases LDL oxidation, and reduces oxidative stress (90–92). Importantly, inhibition of either angiotensin-converting enzyme or the angiotensin receptor attenuates atherosclerosis in animal models (93–95). Studies in humans show that inhibition of the renin–angiotensin system improves endothelial function, reduces oxidative stress, and reduces cardiovascular events (96–99).

Insulin action is impaired along the PI 3-kinase intracellular signaling pathway in insulin resistance. This is the signaling pathway responsible for the metabolic actions of insulin. In contrast, signaling along the ERK 1/2 MAP kinase pathway, which controls many of the mitogenic actions of insulin, remains intact. This selective impairment of the PI 3-kinase signaling branch of insulin action with intact signaling along the MAP kinase pathway has been identified in VSMCs, endothelial cells, and skeletal muscle cells in vitro, in animal models of insulin resistance, and in insulin-resistant humans (22,100–104). Since insulin resistance is accompanied by compensatory hyperinsulinemia, preferential signaling along the mitogenic pathway of insulin action may also contribute to progression of atherosclerosis (105,106).

VSMCs are a key cellular component of atheromatous lesions, and account for a large proportion of total mass (107). VSMCs exhibit two distinct phenotypes that represent extremes along a spectrum of differentiation. Phenotypic modulation is not only central to VSMC function in normal physiology but also in the development of atherosclerosis. The quiescent or fully differentiated phenotype is the usual state of VSMC in the vascular wall, whereas VSMC response to injury is characterized by phenotypic switching to the poorly differentiated synthetic phenotype. Interestingly, the quiescent phenotype is maintained through the presence of insulin, NO, cyclic AMP response-element binding protein (CREB), and other factors, whereas platelet-derived growth factor (PDGF) promotes VSMC proliferation and migration (108–111).

Insulin exerts anti-atherosclerotic effects on VSMCs in normal physiology. For example, insulin enhances eNOS expression with subsequent production of NO, the principal mediator of VSMC relaxation (61). Insulin also contributes to the maintenance of VSMC quiescence, and counteracts the pro-atherogenic effects of PDGF in VSMC (102). However, these aspects of insulin action are mediated via the PI 3-kinase signaling pathway; so it is conceivable that insulin may lose these anti-atherogenic effects in insulin-resistant states. For example, insulin loses its ability to antagonize the effects of vascular endothelial growth factor on ICAM-1 and E-selectin expression in endothelial cells in an in vitro model of insulin resistance (103). We have shown that this model of insulin resistance leads to reduction of insulin's ability to maintain VSMC quiescence and to counteract or reverse the de-differentiating effect of PDGF (102). In contrast to maintenance of VSMC differentiation, intracellular signals responsible for VSMC migration and proliferation appear to involve the Ras–MAP kinase-dependent pathway (102,108,112).

This observation has significant implications for the role of compensatory hyperinsulinemia in the pathogenesis of atherosclerosis progression in patients with

insulin resistance. While the anti-atherogenic effectiveness of insulin is lost in the presence of insulin resistance, its pro-atherogenic potential, mediated largely via MAP kinase-dependent signaling, may be enhanced in the presence of compensatory hyperinsulinemia. This hypothesis is supported by data showing that elevated insulin levels enhance the transcriptional activity of NF-κB induced by angiotensin II, hyperglycemia, and advanced glycosylation end products in VSMCs (113). Furthermore, insulin in the presence of wortmannin, an inhibitor of the PI 3-kinase pathway, increases monocyte interactions with endothelial cells and monocyte arrests (103). The term "monocyte arrest" is used to describe the stopping of a monocyte on vascular endothelium, which is thought to be an initial step in the development of atherosclerosis. Because VSMC migration in response to insulin is MAP kinase dependent, it is expected to occur in the presence of insulin resistance, and may even be enhanced in response to compensatory hyperinsulinemia. VSMCs from a rodent model of diabetes exhibited enhanced expression of pro-inflammatory mediators (114). However, despite numerous studies regarding the regulation of VSMC phenotype, the differentiation and regulation of VSMCs in atherosclerosis are highly complex and not well understood (107).

A potentially important regulator of vascular wall response to injury in atherogenesis and atherosclerosis is the transcription factor early growth response-1 (Egr-1) (115–117). Egr-1 is an 80–82 kD inducible zinc-finger protein that is the prototype of the early growth response gene family. Many stimuli associated with vascular disease, such as hypoxia, physical forces, cytokines, and growth factors including insulin, and oxidative stress, rapidly, yet transiently, induce Egr-1 mRNA in tissue culture (118–122). We have shown that insulin induces expression of Egr-1 mRNA within 1 h via activation of the ERK 1/2 MAP kinase pathway, and increases Egr-1 mRNA levels in rat VSMCs (122). Egr-1 acts as a transcription factor that binds to the proximal promoter region of multiple genes, whose products influence pro-inflammatory vascular response to injury, including IL-1β, tumor growth factor-β, MCP-1, IL-2, ICAM-1, and NF-κB. Furthermore, many of the gene products induced by Egr-1, such as TNF-α (123), also cause further induction of Egr-1 in a feed-forward effect. In animal models of arterial injury, Egr-1 is elevated at sites of vascular injury. In the fibrous cap of human atherosclerotic lesions, Egr-1 mRNA is 5-fold higher than in adjacent tunica media (124). In a mouse model of atherosclerosis, absence of Egr-1 resulted in decreased atherosclerotic lesion complexity and lesion area as well as decreased transcription of inflammatory and prothrombotic Egr-1 target genes (125). However, the specific mechanism of Egr-1 effects on VSMC production of inflammatory target genes in insulin resistance is still unclear.

Therapy

Prevention and treatment of insulin-resistance-associated CVD should be targeted toward cardiovascular risk factors and amelioration of insulin resistance, with its associated metabolic abnormalities. Smoking cessation is a key aspect of reducing

the risk for CVD. Dietary modifications to induce weight loss in individuals who are obese and increasing physical activity are therapies that have the potential to improve many cardiovascular risk factors including blood pressure, lipid abnormalities, visceral fat deposition, and insulin resistance. Pharmacologic approaches targeted at reducing cardiovascular risk include aggressive LDL reduction for both primary and secondary prevention, the use of aspirin, and blood pressure lowering. Therapies directed at attenuating the harmful effects of excessive renin–angiotensin–aldosterone system activation are also effective at reducing cardiovascular risk in individuals with T2DM, especially in the presence of renal impairment.

Strategies are available to prevent the development of diabetes and CVD in high-risk individuals who have impaired glucose tolerance. The prevention of diabetes, in particular, is an extremely important goal as the risk for mortality increases markedly with concomitant diabetes and CVD. A recent meta-analysis shows that the relative risk for CVD is increased by 18% for every 1% increase in glycated hemoglobin among subjects with T2DM (126); so glucose-lowering strategies are also an important component of the treatment of insulin-resistance-associated CVD when diabetes is present.

Lifestyle interventions are the cornerstone of therapy for T2DM and for metabolic syndrome. A weight-loss diet and increased physical activity can lead to decreased insulin resistance and improvements in CVD risk factors. A six-month bout of exercise training in hypertensive older individuals lowers blood pressure, abdominal fat mass, and triglycerides, and improves insulin sensitivity (127). Others and we have shown that calorie restriction leading to weight loss improves LDL, HDL, triglycerides, and fasting and postprandial glycemia in subjects with T2DM or metabolic syndrome (128–130). Several large, randomized controlled trials have shown the benefits of lifestyle intervention on metabolic parameters, and a marked reduction in the incidence of T2DM in subjects with impaired glucose tolerance (131–134). The Finnish Diabetes Prevention Study also demonstrated improved insulin sensitivity in its subjects (135).

The Diabetes Prevention Program (DPP) showed that intensive lifestyle intervention reduces the incidence of metabolic syndrome (136); reduces blood pressure, triglycerides and pro-atherogenic LDL phenotype B; increases HDL; and reduces the risk for diabetes (137,138). A significant decrease in cardiovascular events was not found during the 3-year period of the DPP, but it is probable that a longer period of intervention is required to demonstrate a reduction in rates of cardiovascular events. No published randomized controlled trials are available demonstrating reduced cardiovascular events or mortality with lifestyle intervention in insulin-resistant subjects. However, results from the Look AHEAD trial will help answer the question of whether a long-term weight loss program consisting of calorie restriction and increased physical activity can prevent CVD in individuals with T2DM (139,140).

Among pharmacologic therapies, statins have the strongest clinical trial evidence and greatest effect to reduce CVD risk in individuals with insulin resistance. This class of medications inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key enzyme in the cholesterol biosynthetic pathway. Inhibition of cholesterol biosynthesis upregulates the expression of hepatic LDL receptors, increasing LDL

clearance from the circulation and reducing LDL production. Multiple well-designed clinical trials have demonstrated the effectiveness of statins for both primary and secondary prevention of cardiovascular events (141,142). Most trials in subjects with impaired glucose tolerance and/or diabetes also show that statin therapy reduces the risk for cardiovascular events, in the presence or absence of known coronary artery disease (143–149), although this is not true of all trials (150).

Blood pressure lowering is a key aspect of reducing CVD risk and mortality in patients with diabetes who are also hypertensive (151,152). Angiotensin-converting enzyme (ACE) inhibitors reduce cardiovascular end points in individuals with diabetes and CVD or at least one other cardiovascular risk factor. Strikingly, results of the Heart Outcomes Prevention Evaluation Study showed a 37% reduction in cardiovascular mortality at 4.5 years (153). The beneficial effect of ACE inhibitors seems to extend beyond their blood-pressure-lowering effect in some (154) but not all populations studied (155,156). It remains to be seen whether renin inhibitors will be able to reduce CVD risk in individuals with insulin resistance (157).

The TRoglitazone In the Prevention Of Diabetes (TRIPOD) study was a single-center trial demonstrating that TZD therapy prevented the development of diabetes in women with prior gestational diabetes (158). TZD therapy improves endothelial function in individuals with T2DM (159–162). TZDs also decrease the rate of progression of carotid IMT, a surrogate for atherosclerosis (76,163,164), in T2DM and in insulin-resistant non-diabetic individuals (165). The Diabetes Reduction Assessment with ramipril and rosiglitazone Medication (DREAM) trial included over 5000 subjects with abnormal glucose tolerance or abnormal fasting glucose but no prior CVD. Over the median of 3 years of TZD therapy, there was a significant reduction in the incidence of T2DM, but no difference in cardiovascular event rates (166). Unfortunately, the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) study did not show a reduction in the primary composite end point, which consisted of all-cause mortality, nonfatal myocardial infarction, stroke, acute coronary syndrome, endovascular or surgical intervention in coronary or leg arteries, and above-ankle amputation after nearly 3 years of pioglitazone therapy (75), while the incidence of edema and heart failure were increased. However, there was a reduction in the incidence of the secondary end point, which was a composite of all-cause mortality, nonfatal myocardial infarction, and stroke. Additionally, this trial did not demonstrate a significant reduction in primary or secondary composite end points when the study population was stratified by prior history of stroke (167).

A recent meta-analysis raised concerns about the cardiovascular safety of rosiglitazone (168). This meta-analysis included 42 randomized trials examining the effects of 24 or more weeks of treatment with rosiglitazone on myocardial infarction or deaths from cardiovascular causes in individuals with T2DM. Data from the DREAM trial and from A Diabetes Outcome Prevention Trial (ADOPT) (169) were included in the analysis. Of note, six trials were excluded from the analysis because no events were reported. The rosiglitazone group was found to have an odds ratio of 1.43 (95% confidence interval 1.03–1.98, $p = 0.03$) for myocardial infarction. The odds ratio for death from cardiovascular causes (1.64) was not significant (95% confidence interval 0.98–2.74, $p = 0.06$), although there was a trend toward increased risk in the rosiglitazone group. Since the publication of this meta-analysis,

an unscheduled interim analysis of the Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes (RECORD) trial (170), another meta-analysis of rosiglitazone effects on cardiovascular outcomes (171), a Cochrane Database review of rosiglitazone for T2DM (172), a meta-analysis of pioglitazone effects on cardiovascular outcomes (173), and a meta-analysis examining the effect of TZDs as a class on cardiovascular outcomes (174) have also been published. Several questions have been raised regarding the validity of the initial meta-analysis, and the strength of conclusions drawn from the interim analysis of RECORD, since it was markedly underpowered for the primary outcome (172, 175, 176). The Cochrane Database review concludes that the available trial data do not provide evidence for cardiovascular benefit of rosiglitazone, but are inadequate to conclusively prove that it increases the risk of cardiovascular events (172). Despite the latter point, the authors felt that unless data from ongoing and future trials show convincingly that rosiglitazone does not increase the risk of cardiovascular disease, other glucose-lowering agents should be selected.

Therefore, whether rosiglitazone increases the risk for cardiovascular events is still a concern. Although the Food and Drug Administration (FDA) has not pulled rosiglitazone from the market or stopped ongoing trials, it has added a black-box warning for heart failure to the labeling for rosiglitazone, and recently revised this black-box warning to include a possible increase in cardiovascular risk. Similarly, available primary trial data and meta-analyses for pioglitazone do not show equivocally that this agent lowers cardiovascular mortality in individuals with insulin resistance. The RECORD trial will end in approximately 2010 but another interim analysis will be done before this at the request of the FDA. Action to Control Cardiovascular Risk in Diabetes (ACCORD) will provide more information regarding the cardiovascular safety of rosiglitazone. The Veterans Affairs Diabetes Trial (VADT) may also help answer this question (177), although the main objective of this study is to determine the effect of strict glycemic control on cardiovascular events in T2DM. The results of these trials, along with new well-designed head-to-head comparison trials with well-defined cardiovascular end points, are needed to resolve the concerns regarding rosiglitazone, and to determine whether these safety concerns should be generalized to TZDs as a class.

Acarbose is an alpha-glucosidase inhibitor used as a glucose-lowering therapy, which has been demonstrated to slow the progression of carotid IMT (178). Importantly, it reduces the relative risk for cardiovascular events by 49% in individuals with impaired glucose tolerance (179). Metformin was not shown to reduce cardiovascular events after 3 years of therapy in the DPP (137), but is being studied in the VADT in the context of intensive glycemic control (177).

Future directions

The primary prevention of both diabetes (180) and CVD in diabetes (181,182) is critical for reducing the excess morbidity and mortality that occur with insulin-resistance-associated atherosclerosis. More studies are needed to identify individuals

who should be targeted for pharmacologic therapy. Existing therapies need to be implemented more widely in people who need them. Furthermore, the existing therapies are not completely effective in reducing the risk for cardiovascular events and cardiovascular mortality, so other mechanisms and pathways need to be examined both on a basic level as well as in human studies to reduce the toll of insulin-resistance-associated CVD.

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Chapter 13
The Liver and Insulin Resistance: The Important Convergence of Endocrinology and Hepatology

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Keywords Insulin resistance, fatty liver, metabolic syndrome

Introduction

Recognition of a link between insulin resistance (IR) and liver disease dates back at least 100 years to the term "hepatogenous diabetes", which describes the association between cirrhosis and development of diabetes (1) and more recently to the term "diabetic fatty liver" which antedated the now more common terms "nonalcoholic steatohepatitis" (NASH) and "nonalcoholic fatty liver disease" (NAFLD). These terms were introduced in the 1980s and 1990s, respectively. Since their introduction, the ever-rising prevalence of obesity has brought increased attention to these disorders as the hepatic manifestation of "metabolic" or "insulin resistance" syndrome. Indeed, IR appears to be the common link among metabolic syndrome, obesity, and nonalcoholic fatty liver. Metabolic alterations and hepatic steatosis can develop in the insulin-resistant state in the absence of and prior to diabetes mellitus. Moreover, it is now known that IR correlates with increasing fibrosis in other liver diseases including hepatitis C.

These pathological relationships have raised a now-common issue: "Does insulin resistance cause fatty liver or does fatty liver cause insulin resistance?". Below, we will discuss why the answer to this complicated "either – or" metabolic question is actually 'yes'. In other words, fatty liver both results from IR and contributes to the problem. In order to better understand the relationship between IR and fatty liver disease, it is best to consider them in light of the most basic actions of insulin and other co-variables important in energy homeostasis and, perhaps, to consider the most fundamental disturbance in pathological IR states – disturbed intracellular fatty acid metabolism that leads to "lipotoxicity" or cellular injury due to the excessive accumulation of triglycerides and fatty acids and their subsequent oxidation. By understanding the normal flux of glucose and lipid between the major targets of insulin (adipose, muscle, and liver) and how IR relates to fatty liver disease, we can hopefully identify possibilities for early therapeutic intervention. Moreover, integration of the hepatologist's knowledge of human hepatic pathology and pathophysiology coupled to the endocrinologist's knowledge of insulin signaling and the

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associated metabolism of glucose and fat is essential in managing the growing problem of liver disease associated with the IR syndrome.

Normal Glucose and Lipid Flux in the Fed and Fasting States

The liver plays a central role in maintaining energy homeostasis. It is the major source of glucose production through glycogenolysis and gluconeogenesis. Overall glucose homeostasis occurs through a balance between energy supply and demands from key organs (muscle, adipose, brain, and liver) and differs in the fed versus fasting state. In the fasting state, rates of glucose production and utilization are equal. In this state, 50% of glucose disposal occurs in the brain, 25% occurs in the splanchnic area, and 25% occurs in muscles (2). The liver meets its glucose demands through glycogenolysis, and later through gluconeogenesis if fasting is prolonged beyond 10–18 h. While other organs are able to synthesize and hydrolyze glycogen, only the liver and kidney express glucose-6-phosphatase, the enzyme required for the release of glucose into the circulation.

Adipose tissue stores of triglycerides are an important source of energy during fasting through the release of stored fatty acids by hormone-sensitive lipase (which is normally inhibited in the fed state by insulin). Once cleaved from glycerol, albumin-bound fatty acids are delivered to other tissues via the fatty acid binding protein (FABP), which facilitates movement of fatty acids into the cell. There they can then undergo oxidation to provide adenosine triphosphate (ATP) for cellular activity, while glycerol is either used to re-synthesize triglyceride or is converted to glucose through gluconeogenesis in the liver.

During periods of abundant calorie intake, excess glucose is converted to lipids, which are either stored as triglycerides or incorporated into lipoproteins (i.e., very low density lipoproteins; VLDL) to be exported out of the liver. De novo fatty acid synthesis from dietary carbohydrate occurs primarily in the liver, in lactating mammary glands, and to a lesser extent in the adipose tissue. In the fed state, triglycerides are synthesized within adipocytes through the action of lipoprotein lipase.

Hormonal regulation: insulin, glucagon, and the insulin receptor signaling pathway

Under normal conditions, plasma glucose concentration is tightly maintained despite wide fluctuations in glucose supply and utilization during different states (fasting, fed, exercise, rest). Regulation is achieved through the competing hormones insulin, glucagon, and epinephrine, and is heavily influenced by the activity of adipokines from adipose tissue and, more fundamentally, by the action of increased intracellular fatty acids that alter insulin signaling.

Insulin: Insulin is an anabolic hormone that regulates glucose homeostasis through actions on three integrated target tissues: liver, muscle, and adipose. It stimulates cell growth and differentiation, promotes storage of substrates in liver, fat, and

muscle through lipogenesis as well as glycogen and protein synthesis, and inhibits lipolysis, glycogenolysis, and protein breakdown. Following a meal, one-third of the glucose is delivered to the liver, one-third to muscle and adipose, and one-third to non-insulin-dependent tissues (i.e., brain). A rise in plasma glucose concentration stimulates the release of insulin from pancreatic β-cells. The liver removes 60% of the insulin that enters through the portal vein. Peripheral insulin mediates glucose uptake, glycolysis, and conversion to glycogen in muscle and adipose tissue. Most of the glucose delivered to peripheral tissues is utilized by muscle (80–85%), whereas only a small amount (4–5%) is metabolized in adipose (2). While glucose disposal in adipose tissue is relatively low compared to muscle, adipose tissue plays a key role in overall glucose homeostasis through the release of free fatty acids (FFAs) and expression of adipokines, as will be discussed below.

Insulin regulates glucose metabolism in the liver through both direct and indirect means. Direct effects on the liver result in decreased glycogenolysis and gluconeogenesis by decreasing transcription and suppressing the activity of phosphoenolpyruvate carboxylase (PEPCK) and glucose-6-phosphatase (G-6-Pase), which are key enzymes involved in gluconeogenesis (3). Indirect effects result from insulin's action on peripheral tissues. Insulin causes peripheral uptake of glucose in adipose and muscle tissue by stimulating translocation of the transporter GLUT 4 to the plasma membrane (4). Insulin is anabolic in muscle by promoting glycogen and lipid synthesis while suppressing lipolysis and gluconeogenesis. Insulin promotes triglyceride storage and decreased FFA release in adipose tissue. The combined effects of insulin on muscle and adipose tissue result in decreased FFA influx into liver, which indirectly leads to less gluconeogenesis.

Glucagon and epinephrine: Glucagon is a polypeptide secreted by α-cells of the pancreas. Under physiologic conditions, it acts on the liver by activating glycogenolysis and has a relatively small role in gluconeogenesis. Glucagon favors partitioning of FFAs released by adipose tissue to oxidation in the liver to acetyl CoA, which can be used to form ketone bodies. Stress hormones including epinephrine, cortisol, and growth hormone increase glucose through stimulation of hepatic gluconeogenesis (5). Epinephrine is also especially important in activating adenylate cyclase in the adipocyte membrane, which results in the formation of 3,5′-cyclic adenosine monophosphate (AMP) that leads to lipolysis through activation of hormone-sensitive lipase.

Insulin receptor signaling pathway: The insulin receptor is a tetramer composed of two extracellular α–units bound to two membrane-spanning β-units (Fig. 13.1). Activation begins with binding of insulin to the α–subunit, which then triggers autophosphorylation of the β-subunit. Tyrosine kinase-mediated phosphorylation of the insulin receptor substrate (IRS) then triggers a cascade of pathways that differ depending upon the target tissue and the specific IRS type (6, 7). Four different types of IRS proteins have been identified: IRS 1 (skeletal muscle), IRS 2 (liver), IRS 3 (adipose, β-cells, liver), and IRS 4 (thymus, brain, kidney) (8). Binding of insulin to IRS proteins in muscle and adipose activates phosphatidylinositol 3-kinase (PI3K), which leads to translocation of GLUT 4 glucose transporter proteins to the cell membrane, resulting in an increase in transport of glucose into cells.

Unlike in muscle and adipose tissue, glucose transport in the liver occurs through the GLUT 2 transporter, which is not affected by insulin. This is an

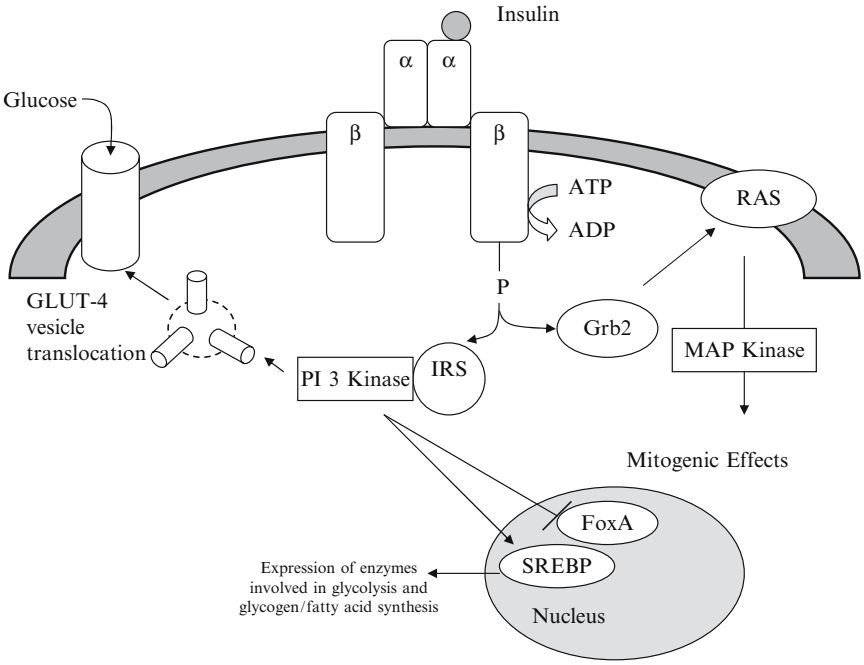

Fig. 13.1 Insulin receptor signaling pathway. Insulin binds to a four-subunit membrane spanning receptor, triggering tyrosine phosphorylation of the beta subunit, which results in a signaling cascade with combined endpoints: (a) translocation of the GLUT-4 glucose transporter; (b) metabolic effects mediated through steroid regulatory element binding protein (SREBP); and (c) mitogenic effects (growth, cell differentiation) through activation of the mitogen-activated protein kinase (MAPK) pathway

important distinction as failure of insulin to suppress endogenous glucose production by the hepatocyte constitutes one of the pillars of the insulin-resistant state. This non-dependence of the hepatocyte on insulin for glucose uptake may in fact allow the liver to function as something of a sink for excess glucose. Insulin signaling by PI3K in the liver appears to be important in activation of downstream expression of genes encoding enzymes involved in glycolysis, glycogen synthesis, and lipid synthesis through steroid regulatory element binding protein (SREBP)-1c. Gluconeogenesis is inhibited by altering gene expression by the forkhead family of transcriptional factors (FoxA), while growth and cell differentiation effects of insulin are mediated through activation of the mitogen-activated protein kinase (MAPK) pathway via growth factor receptor binding protein 2 (Grb2).

The Insulin-Resistant State

Insulin resistance and insulin resistance syndrome defined: IR is defined as impaired response to normal or elevated insulin levels (9). While it is often quantified through the euglycemic clamp model (10) and Homeostasis Model Assessment

(HOMA)(11) or Quantitative Insulin-Sensitivity Check Index (QUICKI) assessments (12, 13), it is important to note that these only address the role of insulin in glucose metabolism. Using a modification of the fasting insulin and glucose-derived QUICKI, efforts have been made to incorporate fasting fatty acid levels to improve the measurement of IR (14). However, all these tests have some inherent limitations, although they remain clinically and experimentally very useful (15).

Insulin resistance syndrome has been defined by Reaven to encompass the multiple sequelae that result from the compensatory hyperinsulinemic state associated with IR. This includes dyslipidemia, endothelial dysfunction, and alterations in procoagulant factors and markers of inflammation (16). Clinical manifestations include diabetes mellitus, cardiovascular disease, hypertension, polycystic ovary syndrome, and NAFLD.

Target organ alterations in IR: With the exception of specific genetic defects in the insulin receptor (leprechaunism, Rabson–Mendenhall syndrome, type A syndrome of IR), IR results from the typical combination of predisposing genetic and environmental factors (e.g., excessive calorie intake for the level of physical activity). In the early stages of IR, compensatory hyperinsulinemia maintains euglycemia. Progression to impaired glucose tolerance and later diabetes mellitus occurs when β-cells of the pancreas are no longer able to provide adequate insulin production. Thus, the development of overt diabetes in insulin resistance syndrome depends on the vitality or lack thereof of the islet cells, and other target organ damage resulting from hyperinsulinemia may occur in the absence of diabetes mellitus.

Phenotypic manifestations of insulin resistance syndrome are characterized by alterations in all three target tissues. In skeletal muscle, IR is associated with decreased glucose uptake (due to impaired translocation of GLUT4), decreased glycogen synthesis, and increased triglyceride accumulation (17). In adipocytes, the major effect of IR is increased lipolysis with uncontrolled release of FFAs (18). Excess FFAs released by adipose tissue plays a role in mediating hepatic IR both directly by interfering with insulin receptor signaling (6), or indirectly by promoting increased hepatic triglyceride accumulation and subsequent hepatic steatosis. Hepatic IR, defined as impaired ability of insulin to suppress hepatic glucose output, can be viewed as a result of impaired response to insulin resulting from mediators released by peripheral tissue (FFAs and adipokines) or from causes within the liver itself (hepatic steatosis). The association of hepatic steatosis with IR may be bidirectional; products released by IR in peripheral tissue (FFAs) contribute to hepatic steatosis; however, hepatic steatosis itself also contributes to IR.

Hepatic steatosis: is fatty liver a cause, a result, or simply a part of IR?

Epidemiologic studies support a direct association between IR and NAFLD (19–21). In non-diabetic individuals, hepatic steatosis correlates directly with IR as measured by HOMA-IR (22). A direct effect of hyperinsulinemia on hepatic

steatosis is supported by the observation of a rim of subcapsular hepatic steatosis when insulin is added to peritoneal dialysate (23) and observation of hepatic steatosis following successful intraportal islet transplantation (24). This provides evidence of a direct effect of hyperinsulinemia on lipogenesis and is supported by the evidence of increased de novo lipogenesis in individuals with NAFLD (25). Insulin promotes lipogenesis through the activation of sterol response element binding protein (SREBP) – a major transcription factor that activates genes involved in lipogenesis (26). Hepatic steatosis associated with IR also results from excess FFAs released by peripheral lipolysis (25, 27).

Whereas there is a clear association between hepatic steatosis and IR, evidence suggesting that hepatic steatosis may itself cause IR is less clear. Evidence in rats with fatty liver in the absence of peripheral IR suggests a direct correlation between hepatic fat accumulation and hepatic IR through stimulation of gluconeogenesis and impaired activation of glycogen synthase (28). In humans, increased liver fat is associated with impairment of insulin-induced suppression of hepatic glucose output, independent of obesity and fat distribution (29). However, hepatic steatosis is not invariably associated with IR. For example, individuals with hepatic steatosis in the setting of familial heterozygous hypobetalipoproteinemia (FHBL) have normal HOMA-IR. In addition, patients with exposure to petrochemicals have been clearly shown to develop fatty liver independent of IR, as measured by HOMA (30).

IR in Disease States

Nonalcoholic Fatty Liver Disease: NAFLD is common among individuals with obesity and IR (31, 32) and its prevalence in the general population is expected to increase with rising obesity (33). Pediatric cases of NAFLD are now being increasingly recognized and are an important public health concern (34). The term NAFLD is used to include both simple hepatic steatosis without inflammation and NASH. The latter is defined by histologic findings of hepatic steatosis, inflammation, hepatocyte ballooning, and fibrosis (35), in conjunction with clinical and historical features, notably the absence of significant (>20 g daily) alcohol intake. Steatosis without inflammation is generally felt to carry a benign prognosis (36), whereas individuals with NASH can progress to cirrhosis and hepatocellular carcinoma (37). IR and its metabolic consequences are the fundamental mechanisms leading to hepatic fat accumulation in NAFLD. However, only a subset of individuals with NAFLD has NASH. A “two-hit” hypothesis has been proposed as a pathophysiologic model to account for cellular injury, inflammation, and fibrosis beyond the simple hepatic steatosis observed in the subset of individuals with NASH (38).

Fat accumulation associated with IR is the “first hit” in NASH and results from a derangement in the physiologic mechanisms designed to maintain energy homeostasis. There is preferential activation of cellular pathways characteristic of calorie/macronutrient deficiency despite being in a state of excess. The liver, skeletal muscles, and adipose tissue all play roles in hepatic steatogenesis and promotion

of IR. The resistance of visceral adipocytes to insulin's anti-lipolysis effects drives excess FFA accumulation. Additionally, escalated hepatic de novo lipid synthesis, reduced fatty acid β-oxidation by hepatocyte mitochondria, and impaired hepatic VLDL export, promote FFA accumulation (39). The cellular mechanisms by which these paradoxical and maladaptive events occur involve a complex interplay between the insulin/glucagon ratio and the cytokine milieu. These factors together modulate the transcription of the relevant genes and activity of their target enzymes. The sterol regulatory element binding protein 1c (SREBP 1c), carbohydrate responsive element binding protein (ChREBP), and peroxisome proliferator activated receptors (PPAR) are three such transcription factors. PPARs promote lipid oxidation via increasing uptake of long-chain fatty acids in skeletal muscle and liver and promoting their β-oxidation in mitochondria. Hepatic lipogenesis is enhanced via SREBP 1c and ChREBP, which are stimulated by insulin and glucose, respectively (40). The biology of SREBP 1c provides a relevant example of the interrelationship between diet, cytokines, and the pathogenesis of NAFLD. Glucose, sterol, and saturated fat consumption upregulates SREBP 1c synthesis, whereas intake of polyunsaturated fats has the opposite effect (41). These nuclear transcription factors are also impacted by cytokines released from adipose tissue, the so-called "adipokines". Adiponectin is an insulin sensitizing adipokine, and animal models have demonstrated inverse correlation between adiponectin and SREBP 1c levels (42). The role of adipokines in NAFLD will be discussed in detail below in the context of obesity and IR.

The evolution of simple hepatic steatosis to NASH is characterized histologically by the appearance of cytologic ballooning, necroinflammation, and pericellular fibrosis. The two-hit hypothesis accounts for this transition by proposing additional insults that result in more severe hepatic manifestations of the metabolic syndrome. The inflammatory component of NASH is partially due to macrophage accumulation in visceral adipose tissue. These macrophages secrete proinflammatory cytokines, which further promote IR, and contribute to trafficking of inflammatory cells to the steatotic liver (43). The ensuing hepatocyte apoptosis results from oxidative stress related to reactive oxygen species (ROS) accumulation and immune-mediated cytotoxicity. Mitochondrial dysfunction promotes ROS formation, resulting in both cellular necrosis and a self-propagating feedback cycle from ROS stimulation of TNF-α synthesis. TNF-α and other pro-inflammatory cytokines potentiate mitochondrial dysfunction and promote the lymphocyte infiltration typical of NASH (40).

The Role of obesity in IR and NAFLD: While obesity is not essential for the development of IR and NAFLD, the strong association among these three conditions may provide some understanding of how the liver, adipose tissue, and other sites of insulin action are related in the development of clinical manifestations observed in the insulin-resistant state. Epidemiologic studies clearly demonstrate a positive correlation between increasing body mass index (BMI) and IR (44, 45). Similarly, a positive association between obesity and NAFLD exists (46, 47). Central to the understanding of how obesity plays a role in IR and NAFLD is the concept that adipose tissue is not only an energy store but also serves a role as an

endocrine organ through the release of circulating FFAs and adipokines. One might even go so far as to think of these circulating factors as part of a "vicious cycle" whereby they are both a contributor to and a result of IR.

Obesity is associated with elevated circulating FFA levels (48, 49) due to increased adipose mass as well as increased lipolysis due to IR. Increased FFA contributes to peripheral IR (50) via impairment of GLUT-4-mediated glucose transport (51, 52) and hepatic IR through competitive inhibition of IRS 2 signaling by diacylglycerol (53). In vitro, FFAs promote hepatic IR by stimulating PEPCK and pyruvate carboxylase (54), key enymes in gluconeogenesis, and by increasing the activity of glucose-6-phosphatase, the enzyme responsible for release of glucose from the liver (55). In vivo, increased serum FFA levels are associated with increased hepatic gluconeogenesis and decreased glycogenolysis (54, 56, 57). Increased FFAs associated with obesity and IR result in hepatic steatosis through increased triglyceride formation, increased de novo lipogenesis, and decreased secretion of apolipoprotein B, which results in decreased export of triglycerides out of the hepatocyte as VLDL (39).

Altered expression of adipokines (leptin, adiponectin, resistin, TNF-α) by adipose tissue in obesity also contributes to IR and hepatic steatosis. Adiponectin is a protein with insulin sensitizing effects that is expressed exclusively by adipocytes in response to PPAR-γ activation. Receptors for adiponectin have been identified in skeletal muscle and liver (58) and are downregulated in obesity-linked IR and diabetes (59). Serum adiponectin levels correlate inversely with BMI (60, 61) and liver fat content (62–64), suggesting an inhibitory role in obesity and hepatic steatosis. Recombinant adiponectin improves IR in mouse models of obesity and type 2 diabetes (65). Insulin sensitizing effects may occur through increased fatty acid oxidation in muscle and decreased fatty acid transport into the liver, resulting in a net decrease in triglyceride accumulation in both muscle and liver (65). Leptin is a protein expressed by mature adipocytes that acts on the hypothalamus to serve as a signal of energy sufficiency. It is produced in proportion to adipose tissue mass and improves IR and hepatic steatosis in patients with severe lipodystrophy (66). Serum leptin levels are increased in NASH (67); however, its role in NASH is debated (68).

Unlike leptin and adiponectin, which are produced exclusively by adipocytes, other adipokines such as TNF-α are derived mostly from macrophages in adipose tissue. Increased TNF-α levels are found in obese individuals owing to increased macrophage infiltration in adipose tissue (69–71) and overexpression of TNF-α by enlarged adipocytes (72). Levels are elevated in individuals with NAFLD compared to controls matched for age, BMI, and sex (73). TNF-α impairs insulin signaling through serine phosphorylation of IRS-1 (74).

Lipodystrophy Syndromes: Paradoxically, loss of adipose tissue, as seen in patients with lipodystrophy, is also associated with IR. Lipodystrophies are disorders characterized by selective and variable loss of subcutaneous adipose tissue. They are clinically heterogeneous, and the affected patients are predisposed to IR, hypertriglyceridemia, hepatic steatosis, polycystic ovary syndrome, and type 2 diabetes. Lipodystrophies can be either acquired or inherited (familial).

Acquired lipodystrophies are much more common than the inherited forms. The most common form of acquired lipodystrophy is that seen in patients with human immunodeficiency virus (HIV) infection who are receiving treatment with highly active protease inhibitors. Patients typically present with loss of subcutaneous fat in the face, arms, and legs (75), with or without concomitant fat accumulation in the neck and trunk (76). Patients may develop IR, hypertriglyceridemia, hepatic steatosis, low serum levels of HDL cholesterol, and hyperglycemia (77–79). Possible mechanisms by which protease inhibitors cause lipodystrophy include impaired pre-adipocyte differentiation (80), increased apoptosis of subcutaneous adipocytes (81), and reduced mRNA expression of sterol regulatory element binding protein 1c (SREBP1c) and peroxisome proliferator activated receptor γ (PPARγ), two key transcription factors involved in adipogenesis (82). In addition, protease inhibitors may directly induce IR by reducing the intrinsic transport activity of glucose transporter 4 (83).

Other forms of acquired lipodystrophies are rare (84). Patients with acquired generalized lipodystrophy present with clinical features of loss of subcutaneous fat, muscular prominence, acanthosis nigricans, hepatic steatosis, autoimmune hepatitis, and cirrhosis. Patients with acquired partial lipodystrophy have fat loss affecting the face, neck, arms, thorax, and upper abdomen. In contrast, excess fat may be deposited in the hips and legs. Both acquired generalized and partial lipodystrophies occur during childhood and adolescence and occur approximately 3–4 times more often in women. Localized lipodystrophies refer to loss of subcutaneous adipose tissue in small areas and may be caused by local injection of medicines such as insulin and corticosteroids, recurrent pressure, trauma, inflammation, or other unknown mechanisms.

Inherited lipodystrophies are extremely rare and are caused by various genetic mutations (84). Congenital generalized lipodystrophy is an autosomal recessive disorder characterized by near-complete lack of adipose tissue since birth with clinical features including acanthosis nigricans, hepatic steatosis, cirrhosis, splenomegaly, and umbilical hernia. Patients usually have severe IR/hyperinsulinemia, hypertriglyceridemia, and type 2 diabetes. Familial partial lipodystrophies are characterized by partial loss of subcutaneous fat in various parts of the body with distinct clinical features due to different genetic mutations. Patients may develop hypertriglyceridemia, fatty liver, and type 2 diabetes.

Metabolic complications such as IR, hypertriglyceridemia, hepatic steatosis, and type 2 diabetes increase in frequency and severity with the extent of fat loss. Patients initially develop compensatory hyperinsulinemia and later overt hyperglycemia and type 2 diabetes owing to gradual loss of β-cell function resulting from islet amyloidosis and cell atrophy (85). Though the underlying mechanisms remain unclear, it appears that ectopic accumulation of triglycerides may be the major culprit. Indeed, a major function of subcutaneous adipose depot is to store triglycerides during energy excess, and in patients with lipodystrophies the storage capacity decreases or even disappears, leading to the accumulation of excess triglycerides in the liver, intra-abdominal fat depot, and skeletal muscles (86–88).

A large body of evidence has confirmed that accumulation of fat in these sites leads to IR (89–92). Mice with congenital lipodystrophy manifest with severe IR in the liver and muscle, and hyperglycemia (93). These mice have higher intracellular fatty acyl-CoA content in both muscle and liver than wild-type mice, and have defects in the insulin activation of IRS-1 and IRS-2 associated PI 3-kinase activity in muscle and liver (94). The importance of having subcutaneous fat depots is further demonstrated by the observation that transplanting adipose tissue from wild-type mice into the subcutaneous space in lipodystrophic mice reduces liver and muscle lipid contents and reverses IR as manifested by increased muscle glucose uptake and suppressed hepatic glucose production in response to insulin (94).

Patients with severe lipodystrophy have low plasma leptin levels. In mice with congenital lipodystrophy, chronic low-dose leptin treatment reverses IR and diabetes mellitus (95). This suggests that leptin deficiency may play an important role in the pathogenesis of IR and type 2 diabetes in patients with lipodystrophies. Indeed, recent studies have shown that leptin replacement in these patients results in improved insulin sensitivity in both muscle and liver and better glycemic and lipemic control (66, 96, 97). Reduced intramyocellular and liver fat contents and reduced appetite could be part of the mechanism (66, 96).

Hepatitis C: IR and the associated steatosis is an emerging aspect of chronic hepatitis C infection. Worsening IR affects chronic hepatitis C in several respects. For example, there appears to be an increased prevalence of type 2 diabetes in hepatitis C patients, especially with increasing age, even when adjusting for confounding variables such as weight (98), and an association between increased HOMA and chronic genotype 1 HCV infection has been observed (99). The occurrence of IR and the development of steatosis have a significant impact on the risk of disease progression (100–102). Finally, IR appears to have an impact on the response to interferon-based antiviral therapy, although this aspect remains somewhat uncertain, as it is unclear whether this represents the effects of obesity on drug delivery (103) or direct effects of IR itself. Moreover, more recent studies have failed to confirm results of earlier reports regarding the impact of steatosis and BMI on response, although the association with more advanced histology seems consistent among studies (104). It is possible and even likely that some of these relationships are obscured by the tendency of NASH to become decreasingly steatotic as the disease progresses, and that fibrosis stage, which appears to be accelerated by steatosis in HCV, is also a predictor of sustained virological response. This may explain why another recent study revealed an association between steatosis and stage-3 fibrosis but not stage-4 (cirrhosis) fibrosis (105).

Steatosis when present in HCV is, indeed, often associated with typical findings of NASH in these patients and with increased activation of the hepatic stellate cells (106). The mechanisms by which an HCV-infected liver accumulates excessive triglyceride stores are related to both host and viral factors. Not surprisingly, many such patients have independent risk factors for metabolic syndrome and therefore for NAFLD. However, there are also direct viral replication factors related especially to the metabolism of the nucleocapsid core protein and related to (but not

restricted to) certain genotypes of the virus. For example, the prevalence of steatosis in genotype 3 is almost 2 times that of other genotypes (107). However, both in vitro and in vivo data have indicated that the core protein of other genotypes, including genotype 1, the most common genotype in the United States, alters intracellular fat metabolism (108). These changes appear to involve significantly decreased levels of PPARα and CPT-1, which therefore inhibit fatty acid oxidation. Other mechanisms may be simultaneously at work, including the indirect effects of increased TNFα in HCV and HCV-mediated changes in IR phosphorylation (109, 110). The potential role of insulin sensitizing agents in conjunction with anti-HCV therapy and related concerns about lipid-lowering agents in HCV are areas in need of further investigation (111).

Summary and Conclusions

Insulin regulates energy homeostasis through its effects on key target organs: liver, adipose tissue, and muscle. Glucose and lipid metabolisms are closely linked via circulating FFAs and adipokines and their effects on insulin receptor signaling, glucose transport, and triglyceride accumulation within these organs. Paradoxically, IR is found in states associated with both adipose excess (obesity) and adipose loss (lipoatrophy). Adipokines released from both adipocytes and macrophages within adipose tissue play key roles in mediating IR and in inflammation. Further understanding of the complex interaction between key target organs and circulating mediators of IR may help guide therapy for NAFLD and other clinical manifestations of the insulin resistance syndrome.

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Chapter 14
Polycystic Ovary Syndrome and Metabolic Syndrome

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The polycystic ovary syndrome (PCOS) is a very common disorder with important short-term and long-term consequences. Indeed, affected women manifest many clinical and biochemical features of metabolic syndrome, putting them at increased risk for diabetes and cardiovascular diseases. In the past 15 years, the key role of insulin resistance (IR) in the pathogenesis of PCOS has been stressed, and this common etiology between the two syndromes might account for their similarities. In fact, PCOS is considered a consequence of IR in the same way as metabolic syndrome, and women at risk for PCOS are also subject to the development of metabolic syndrome. Therefore, this chapter will explore the relationships between the metabolic syndrome and PCOS.

Overview

PCOS affects 6 – 10% of women of childbearing age and represents the most frequent medical cause of female infertility (1,2). Currently, it is the most common endocrinopathy among young women and a major general health issue. PCOS is defined by the presence of two of the following three criteria: oligo- or anovulation, hyperandrogenism (clinical and/or biochemical), and polycystic ovaries (3). Other hyperandrogenic disorders must be excluded (congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome).

In the past 15–20 years, the key role of IR and subsequent hyperinsulinemia in the pathogenesis of PCOS has been underscored. New treatment strategies for the syndrome have emerged from this association, but also a concern that women with PCOS could be at higher risk of developing other insulin-resistance-related disorders. This chapter will explore the clinical and pathophysiological connections between PCOS and the metabolic syndrome.

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IR and PCOS

The literature of the last 20 years has demonstrated the presence of IR and compensatory hyperinsulinemia in most PCOS women (1, 2). Using insulin–glucose clamp techniques, Dunaif et al. (4) demonstrated the relationship among IR, PCOS, and obesity (Fig. 14.1). They found that lean control women had the highest insulin sensitivity, while obese PCOS women were the most insulin resistant. As for obese control and lean PCOS women, they had equal insulin sensitivity. The authors attributed these results to a form of IR related to adiposity, as well as another form that was intrinsic to the syndrome. Accordingly, Dunaif and colleagues (5) described an insulin receptor or post-receptor signal transduction defect in PCOS women. Later, they demonstrated increased insulin receptor serine phosphorylation in PCOS women in vitro, which could account for the post-binding defect of insulin action (6). Other studies in vitro using adipocytes from non-obese PCOS women showed insensitivity to inhibition of lipolysis by insulin and a decrease in adipocyte glucose uptake (7), two features consistent with an insulin receptor or post-receptor defect. It has also been shown that PCOS IR is not global, since PCOS women do not show resistance to insulin actions on ovarian androgen production and liver sex hormone binding globulin (SHBG) suppression. Insulin acts directly on the ovary to stimulate androgen biosynthesis, via its own receptor, as demonstrated by multiple theca cell

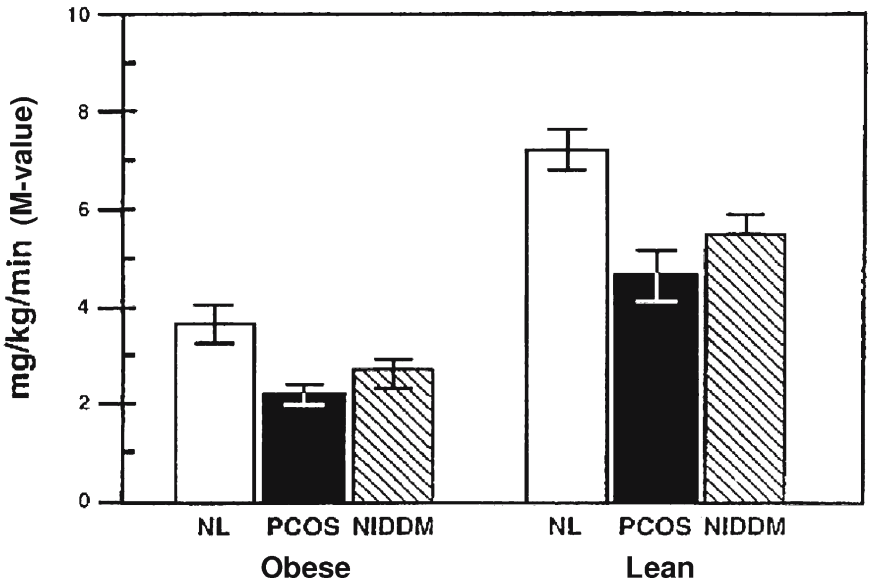

Fig. 14.1 Insulin sensitivity in women with polycystic ovary syndrome (PCOS) and non-insulin-dependent diabetes mellitus (NIDDM). Insulin sensitivity was determined using the euglycemic hyperinsulinemic clamp technique. M-value: glucose infusion rate during the last 30 min of the clamp (mg/min) divided by the weight (kg); NL: normal controls. (Adapted from Dunaif et al. (106), with permission)

studies in vitro (8–10). Nestler et al. (11) compared theca cells from PCOS and control women and demonstrated a greater androgen production in response to insulin in theca cells of PCOS women. This finding was later confirmed by others (8, 12). Insulin also inhibits SHBG production by the liver (13, 14), thereby increasing free testosterone. Notably, insulin sensitizers increase SHBG levels in PCOS women (15, 16). Insulin may also increase ovarian responsiveness to luteinizing hormone (LH) stimulation (17) and pituitary release of LH (18). By increasing ovarian responsiveness to LH and intra-ovarian androgen production, insulin could cause premature activation of preovulatory ovarian follicles, and arrest of subsequent follicles (19). Arrested follicles provide a constant supply of androgens, since atretic follicles are deficient in aromatase. Taken together, all these effects of excessive insulin action lead to hyperandrogenism.

It is important to note that whereas many women present with hyperinsulinemic IR, including most obese women, only very few of them develop PCOS. Consequently, there must be other factors to explain the development of the syndrome. Furthermore, some PCOS subjects are normo-insulinemic and normally insulin sensitive (20–22), and have been shown to normalize their hyperandrogenemia with diazoxide, a pure insulin-lowering medication (Fig. 14.2) (23), as well as insulin-sensitizing drugs

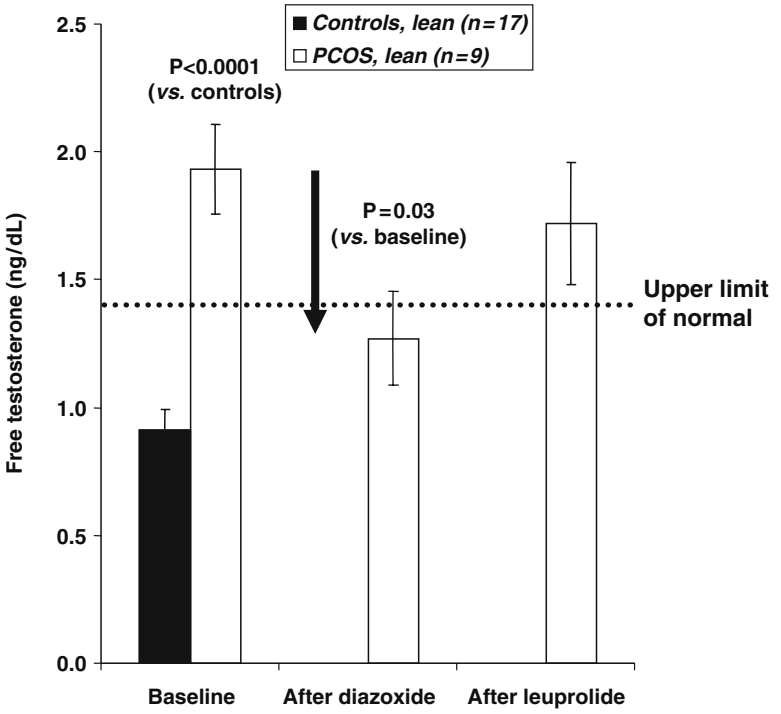

Fig. 14.2 Calculated free testosterone levels at baseline, and after treatment with diazoxide and leuprolide acetate in lean control (bar to the left, $n = 17$) and lean PCOS women (bars to the right, $n = 9$). Results are presented as means with standard errors of the mean (SEM). (Adapted from Baillargeon et al. (23), with permission)

(metformin and rosiglitazone) (24). Therefore, insulin action plays a role in the pathogenesis of PCOS, even in women with normal insulin levels and normal insulin sensitivity, suggesting hyper-responsiveness to insulin actions on androgen production and/or binding. Thus, some PCOS women may present with an increased sensitivity of androgenic pathways to insulin that is severe enough to cause the syndrome even in the absence of systemic resistance to insulin action (25).

However, in most PCOS women, this insulin sensitivity defect is probably not severe enough to be manifest at normal insulin concentrations and they must develop global IR with compensatory hyperinsulinemia before PCOS becomes clinically apparent. Therefore, the association of PCOS with IR may predominately reflect selection based on clinical presentation. According to this hypothesis, the IR characteristic of PCOS might not be specific, but, instead, shares the same causes as other insulin-resistant states. These might be genetically determined or acquired, following a deleterious lifestyle and/or weight gain.

As IR is a key factor in the pathogenesis of PCOS and is highly inheritable (26), along with PCOS (27, 28), siblings of women with PCOS should be more affected by IR and metabolic syndrome than the general population. Accordingly, Baillargeon et al. (29) found that when brothers of women with PCOS are compared to control men, they are characterized by reduced insulin sensitivity (determined by insulin–glucose clamp techniques), decreased glucose tolerance, hypertriglyceridemia, and dyscoagulability, all of which are independent of the degree of adiposity. Other studies found decreased insulin sensitivity in sisters of women with PCOS, presenting the PCOS phenotype or hyperandrogenemia with normal menses (30). Yildiz et al. studied first-degree relatives of PCOS women and noted abnormal glucose tolerance, IR, and hyperandrogenemia compared to normal controls (31). Therefore, siblings of PCOS women might also inherit IR and metabolic syndrome typical of PCOS.

Metabolic syndrome and PCOS

Much overlap exists between PCOS and metabolic syndrome, both clinically and biochemically (Fig. 14.3) (32). According to the 2001 National Institute of Healths' National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) (33), the diagnosis of the metabolic syndrome in women can be made when three or more of the following metabolic abnormalities are present: central obesity with a waist circumference >88 cm (35 in.); fasting serum triglyceride ≥ 150 mg/dl (1.7 mmol/l); serum high density lipoprotein (HDL) cholesterol <50 mg/dl (1.3 mmol/l); blood pressure $\geq 135/85$ mmHg or anti-hypertensive medication; and fasting serum glucose ≥ 110 mg/dl (6.1 mmol/l), 2-h glucose levels after 75-g glucose challenge ≥ 140 mg/dl (7.8 mmol/l), or known type 2 diabetes.

Assuming IR is a central factor in the pathogenesis of the two disorders (2, 34), it is not surprising to find an increased prevalence of metabolic syndrome in PCOS women and a high rate of PCOS in women with metabolic syndrome. Clinically,

women presenting with both PCOS and the metabolic syndrome were found to have more pronounced hyperandrogenemia and increased frequency of acanthosis nigricans (35). These findings suggest a greater degree of IR in those cases. In this section, we will discuss the relationship between PCOS and the metabolic syndrome, as well as each of its components. Table 14.1 summarizes the prevalence of these aspects in women with PCOS.

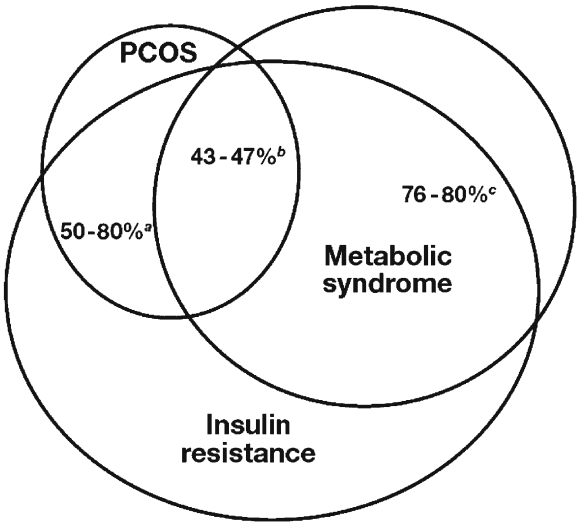

Fig. 14.3 Relationships between the polycystic ovary syndrome (PCOS), the metabolic syndrome, and IR. Approximately 50 – 80% of PCOS women are insulin resistant and 43 – 47% have metabolic syndrome. Similarly, approximately 68 –74% of individuals with metabolic syndrome have IR. (Adapted from Essah et al. (32), with permission)

Table 14.1 Prevalence of metabolic syndrome and its components in women with polycystic ovary syndrome (PCOS)

Characteristics	Prevalence in PCOS (%)	References
Metabolic syndrome	43–46	(35, 36)
Obesity	50–67	(35, 42)
Dyslipidemia	Low HDL-C 68	(32)
	High TG 35–56	(35, 36)
Hypertension	45–70	(35, 36)
Diabetes	7.5–10	(57–59)
Glucose intolerance	31–35	(57–59)

BMI : body mass index; HDL-C : cholesterol concentration contained in high density lipoprotein; TG : triglycerides

Overlap between metabolic syndrome and PCOS

Metabolic syndrome is overrepresented among women with PCOS. Indeed, Glueck et al. (36) studied 138 consecutive women presenting with PCOS using the NCEP-ATPIII criteria and found that the prevalence of metabolic syndrome was 46% among these women.. In a similar study of 161 PCOS women, Apridonidze et al. reported a prevalence of metabolic syndrome of 43% (35). Thisis more than twice the prevalence in a female population of similar age (NHANES III). Another study, by Dokras et al. (37), compared 129 PCOS women with age-matched controls and found an 11-fold increase in the prevalence of metabolic syndrome among women with PCOS (47.3% vs. 4.3%). Major predictors of the metabolic syndrome in PCOS are elevated serum-free testosterone and low SHBG (32), an observation that underscores the causal relationship between metabolic syndrome and the manifestations of PCOS.

The overrepresentation of metabolic syndrome in PCOS was shown to be present in every age category, even in the extremity of age (37). Accordingly, Vural et al. (38) found a prevalence of metabolic syndrome of 11.6% in PCOS women aged between 18 and 22 years, compared to 0% in controls. On the other hand, Margolin et al. (39) showed that 86% of post-menopausal women with an antecedence of PCOS presented the characteristic dyslipidemia of metabolic syndrome as well as a significant increased prevalence of type 2 diabetes and central obesity. Even though obesity partly explains the presence of metabolic syndrome among PCOS women, and the prevalence of metabolic syndrome is much higher in overweight and obese PCOS women, Dokras et al. (37) showed that the difference of metabolic syndrome prevalence between PCOS and controls remains significant even after adjusting for age and body mass index (BMI).

It has also been shown that the prevalence of metabolic syndrome is increased among first-degree relatives of women with PCOS. As noted previously, Sam et al. (30) noted a higher prevalence of metabolic syndrome in sisters presenting the phenotype of PCOS (52%) or hyperandrogenemia with normal menses phenotype (23%), as compared with unaffected sisters (7%). These authors also reported that the prevalence of metabolic syndrome was increased in obese mothers of women with PCOS as compared to obese women from the National Health and Nutrition Examination Survey III (NHANES III) (40). These findings suggest that both conditions track in families and might then be of the same genetic origin.

Conversely, PCOS is also overrepresented in women with metabolic syndrome. In a cross-sectional study of normal women, Korhonen et al. (41) found that women with metabolic syndrome had a significant increase in the serum-free androgen index, along with lower insulin sensitivity, suggesting PCOS or PCOS-like phenotype.

Obesity and PCOS

Approximately 50% of women with PCOS are obese, and their obesity pattern tends to be android (42). This visceral distribution of obesity results in a high waist-to-hip ratio and is more frequently associated with IR. Of note, the android

pattern of fat distribution has been reported even in lean PCOS women (43). Obesity per se is associated with hypertension, dyslipidemia, IR, increased platelet activation, and premature death (44–47).

Dyslipidemia and PCOS

Low serum HDL cholesterol is the most frequently occurring component of metabolic syndrome among women with PCOS and occurs in 68% of the cases (32). It has also been demonstrated that PCOS women present significantly increased low density lipoprotein (LDL) cholesterol when compared with controls (48, 49). Moreover, they frequently present an abnormally high level of small, dense LDL particles and high level of triglycerides-rich lipoproteins (50), a profile known to be atherogenic (51). Notably, dyslipidemia occurs both in lean and overweight PCOS women, and affects all age groups (49). Furthermore, Sam et al. (30, 40) demonstrated increased LDL cholesterol in mothers and affected sisters of women with PCOS, consistent with an inheritable trait.

Hypertension and PCOS

Sustained hypertension is not a universal feature in women with PCOS and seems to occur later in life. However, some studies reported an increased prevalence of hypertension and pregnancy-induced hypertension in PCOS women (52, 53). When compared with controls, postmenopausal PCOS women have a 2.5-fold increased risk of hypertension (54). This late appearance of hypertension, frequently after menopause, might be partly due to the effects of estrogen on the renin–angiotensin–aldosterone axis. Indeed, estrogen blocks the conversion of angiotensin I to angiotensin II and decreases the sensitivity of angiotensin receptors (55). Another factor that plays a role in delaying hypertension is the action of estrogen on nitric oxide synthase which promotes vasodilatation (56). Following menopause, these protective effects are lost.

Diabetes and PCOS

With the implication of IR in the pathogenesis of PCOS, it is not surprising to find an increased prevalence of impaired glucose tolerance and type 2 diabetes among women with PCOS. Indeed, prospective studies have found that the prevalence of impaired glucose tolerance and type 2 diabetes were 31–35% and 7.5–10%, respectively, among women with PCOS (57–59). These numbers are much higher than in the general female population of comparable age, where the prevalence of glucose intolerance and diabetes was estimated at 7.8% and 1%, respectively (60). In addition,

both lean and obese PCOS women have an increased risk of diabetes, although obesity and PCOS have an additive effect on glucose tolerance (59). Progression from impaired glucose tolerance to diabetes is also increased 2- to 5-fold in the PCOS population (61, 62). In the Nurse Health Study (NHS), where more than 100,000 healthy female nurses were followed for approximately 8 years, the development of type 2 diabetes was twice as frequent in women with oligomenorrhea, a good marker of PCOS, as compared to controls, an effect that was independent of weight (63). Furthermore, PCOS women tend to develop diabetes at an earlier age than normal women (5).

The prevalence of PCOS is also increased among women with type 2 diabetes. Indeed, in two retrospective studies of premenopausal women with type 2 diabetes, 27% of women had PCOS (64) and 82% had polycystic ovary on transvaginal ultrasound (65). Thus, PCOS is probably present in more than one-quarter of all type 2 diabetic women.

Cardiovascular risk and PCOS

As shown previously, PCOS is associated with several cardiovascular risk factors, including hypertension, dyslipidemia, obesity, and abnormal glucose tolerance. However, it is also associated with endothelial dysfunction and altered coagulation parameters, implying increased vascular oxidative stress, increased hemostasis, and dysfibrinolysis (51). Moreover, IR is associated with increased levels of serum inflammatory mediators (66, 67), which are implicated in the pathogenesis of cardiovascular disease.

Women with PCOS present with high levels of tumor necrosis factor-α (TNF-α) (68), C-reactive protein (CRP) (69–72), plasminogen activator inhibitor-1 (PAI-1) (73, 74), tissue plasminogen activator (t-PA) (75), and endothelin-1 (76). Endothelial dysfunction is also more common in PCOS women (72, 76, 77), even if they are young, of normal weight, and lacking other cardiovascular risk factors (78). Therefore, it is not surprising to find many studies demonstrating early signs of atherogenesis and coronary heart disease in women with PCOS (38, 79–82). Indeed, many small studies have reported diastolic dysfunction, left ventricular hypertrophy, and increased arterial stiffness in PCOS (51, 78). In the first cohort of the NHS, women with highly irregular menses (an excellent marker of PCOS) displayed a risk of developing coronary artery disease, which was significantly increased by 50% (83). A case–control study found that the prevalence of coronary artery disease was 4 times higher in perimenopausal women with a previous diagnosis of PCOS, as compared to normal controls (21% vs. 5%) (84).

Of interest, this last study did not find any difference in BMI or traditional risk factors of cardiovascular diseases between the groups. Furthermore, Vural et al. (38) reported increased intima-media thickness even in young non-obese PCOS women between 18 and 22 years of age. These results suggest that PCOS might increase the risk for cardiovascular diseases independently of individual components

of the metabolic syndrome, perhaps because of the underlying IR or another factor predisposing women both to PCOS and atherogenesis, such as increased androgenic and other non-metabolic insulin actions. However, no adequate prospective study had demonstrated definitive evidence for an increased cardiovascular risk in PCOS women independent of metabolic syndrome.

Obstructive sleep apnea and PCOS

Obstructive sleep apnea has been associated with IR syndrome (88, 89). It is interesting to note that women with PCOS have a higher prevalence of obstructive sleep apnea than weight- and age-matched controls (90). Notably, it was demonstrated that insulin levels and impairment in glucose tolerance are strongly correlated with the apnea–hypopnea index in PCOS women (91). Sleep apnea is also associated with other features of PCOS, namely increased inflammatory cytokines, interleukin-6 (IL-6), and TNF-α, and with visceral fat (89).

Metabolic syndrome screening in PCOS women

Screening for type 2 diabetes with an oral glucose tolerance test (OGTT) among women with PCOS is well accepted, and is supported by the American College of Gynecology, as well as the American Association of Clinical Endocrinologists (92,93). However, it is not yet recommended by the American Diabetes Association, which recommends an OGTT in PCOS women only when fasting plasma glucose (FPG) is over 5.6 mmol/l (100 mg/dl). Gagnon et al. (94) assessed the predictive value of the FPG cut-off point of 5.6 mmol/l for detection of abnormal glucose tolerance in PCOS women and found a specificity of 98.7%, but a sensitivity of only 48% (Fig. 14.4). With this screening guideline, for every six PCOS women screened only with FPG, one would be missed with an abnormal glucose tolerance. Therefore, these authors recommended screening all women with PCOS using OGTT. In the past few years, many authors have also recommended screening for other components of metabolic syndrome in women with PCOS (32, 38, 51) Accordingly, it was suggested at the Rotterdam European Society for Human Reproduction & Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) meeting that all obese PCOS women should be screened for the different aspects of metabolic syndrome. Since it was demonstrated that even lean women with PCOS have an increased risk of metabolic syndrome, it may be appropriate to extend the screening for metabolic syndrome to every woman presenting with PCOS.

Early screening for abnormal glucose tolerance and metabolic syndrome could prompt early initiation of lifestyle and/or insulin-sensitizing therapy in order to improve both PCOS and associated metabolic disorders and to prevent long-term complications. Furthermore, it could guide clinicians to avoid treatments that may

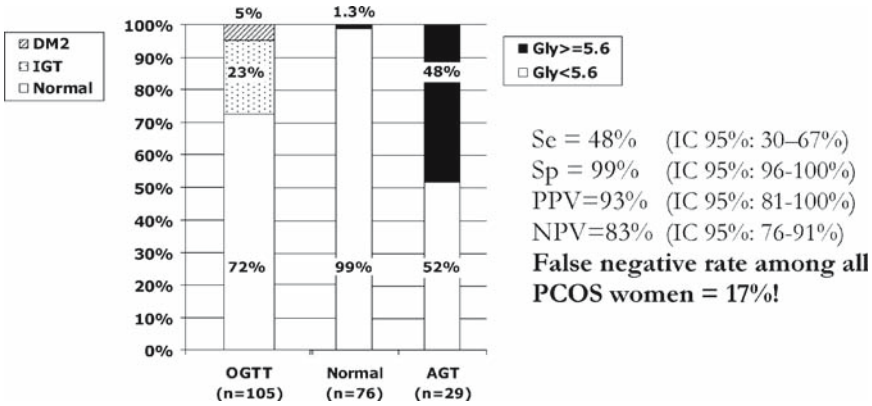

Fig. 14.4 Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the fasting plasma glucose cut-off value of 5.6 mmol/l for the screening of abnormal glucose tolerance (AGT). DM2: type 2 diabetes mellitus; IGT: impaired glucose tolerance; and OGTT: oral glucose tolerance test. (Adapted from Gagnon et al. (94), with permission)

lead to an aggravation of the metabolic syndrome, such as the use of oral contraceptives (see below). If the results are normal, screening again at 2–3 year intervals is probably indicated, since normal results do not preclude future appearance of metabolic syndrome.

As for the screening for PCOS in women presenting the diagnosis criteria of metabolic syndrome, it seems interesting mainly for premenopausal women, when PCOS symptoms are still present and might require management. In the peri- or postmenopausal period, when PCOS symptoms are usually vanishing, the finding of previous clinical PCOS might indicate a more profound IR that could necessitate a more aggressive approach to the cardiovascular risk factors. However, the effectiveness of such screening has not been proven.

Treatment

The mainstay of treatment for both metabolic syndrome and PCOS relies on lifestyle modification. Weight loss, exercise, as well as diet modification and avoidance of toxic substances (cigarette, alcohol, and drugs) have direct effects on IR and cardiovascular risk (95–99). An appointment with a nutritionist for basic advice is also helpful. Moderate weight loss of 5 – 10% of total body weight should be encouraged because it is realistic and it induces a significant decrease in visceral adipose tissues (30% loss) with important benefits on IR (97, 100).

When lifestyle intervention fails to normalize PCOS symptoms or cardiovascular risk factors, or if symptomatic complaints require rapid intervention, pharmacologic treatment needs to be considered. The typical treatment of PCOS has been oral contraceptives (OCs), when fertility is not an issue. Indeed, OCs efficiently treat PCOS

symptoms, including menstrual irregularities, acne, and hirsutism while ensuring a reliable, reversible contraceptive method. However, long-term metabolic complications of the syndrome are receiving growing attention and, since OCs appear to decrease insulin sensitivity and glucose tolerance (2), OCs might not be the optimal treatment for obese and overweight PCOS women. Moreover, evidence for potentially increased cardiovascular risks in women using OCs is growing (2, 101).

Recently, much attention has been given to the use of insulin sensitizers in PCOS, namely biguanide metformin and the thiazolidinediones (TZDs) rosiglitazone and pioglitazone (1,2). In addition to treating oligo-anovulation, fertility, menstrual irregularities, and hirsutism in most women with PCOS, insulin sensitizers also improve glucose intolerance and other cardiovascular risk factors, such as triglycerides levels, blood pressure, and endothelial dysfunction (1,2). Indeed, metformin has been shown to reduce cardiovascular events in obese type 2 diabetic subjects (102). Therefore, even though insulin sensitizers are not considered as first-line therapy in every woman with PCOS, they should be strongly considered when significant risk factors for diabetes or cardiovascular disease are present. One way to assess such risk factors is to determine the concomitant presence of metabolic syndrome, as defined by NCEP-ATPIII guidelines, for example. The effects of insulin sensitizers and OCs as monotherapy for the management of PCOS are summarized in Table 14.2.

Insulin-sensitizing drugs could therefore be used alone as a metabolically favorable alternative to OCs when contraception is not required, or in combination with OCs when contraception is desired. They could also be considered as adjunct therapy in women presenting metabolic complication following the use of OCs. Although few studies directly assessing a combination of insulin-sensitizing drugs and OCs have been published, the beneficial effects of these agents seem complementary, with insulin sensitizers appearing to counteract the deleterious effects of OCs (103, 104). Finally, if dyslipidemia and hypertension are refractory to lifestyle and/or insulin-sensitizing management, adequate antihypertensive and hypolipemic drugs should be introduced following established recommendations (33, 105).

Table 14.2 Effects of insulin sensitizers and oral contraceptives on different aspects of polycystic ovary syndrome

	Insulin sensitizers	Oral contraceptives
Infertility	++	?
Menstrual irregularity	++	+++
Hirsutism	+/++	++
Acne	+	++
IR and features of the metabolic syndrome	+++	–
Glucose tolerance or control	+++	–
Prevention of type 2 DM or cardiovascular diseases	++	–
Prevention of endometrial cancer	+/++	++

+++: very important effect, ++: important effect, +: modest effect, ?: unknown effect, –: no positive effect; DM: diabetes mellitus.

Conclusion

PCOS and metabolic syndrome present many common features, and the implication of IR in the pathogenesis of both syndromes partly explains the overlap. Since, in most cases, IR and compensatory hyperinsulinemia are required for the appearance of PCOS, most PCOS women are also at increased risk for development of metabolic syndrome. Indeed, PCOS and metabolic syndrome may be two alternate clinical presentations of the same underlying pathophysiology, reflecting IR and/or abnormal insulin actions. Accordingly, PCOS is sometimes considered a female expression of the IR syndrome (57).

Since PCOS women are at increased risk of developing cardiovascular disease and type 2 diabetes, lifestyle modifications should be promptly instituted and promoted for life. When lifestyle measures fail or clinical symptoms dictate rapid intervention, concomitant use of insulin sensitizers should be considered and might be preferred to OCs, especially if contraception is not required.

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Part III
Treatment

Chapter 15
Effects of Exercise in Metabolic Syndrome and Diabetes: A Central Role for Insulin Sensitivity

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Keywords exercise, physical activity, T2DM, metabolic syndrome, insulin sensitivity, insulin resistance, cardiovascular risk, endothelial dysfunction, inflammation, cardiac dysfunction, AMPkinase

Introduction

Poor physical fitness is associated with increased morbidity and mortality. It has been observed consistently that low cardiorespiratory fitness and physical inactivity predict mortality in both normal-weight and obese men, in older men and women, and in men with type 2 diabetes mellitus (T2DM) (1–9). Sedentary behavior has been clearly implicated as a factor leading to the development of diabetes, as well as the worsening of cardiovascular (CV) outcomes in patients with diabetes. Physical inactivity has become so common that one group has coined the term "Sedentary Death Syndrome" (10). The sedentary death syndrome model proposes that evolution favored genes that support the physical activity required for long-term health in an agrarian society and that sedentary behavior is maladaptive.

Physical activity/exercise is recognized as a cornerstone of the treatment of patients with T2DM. Over 80 years ago, Allen and others reported that a single bout of exercise lowered the blood glucose concentration of persons with diabetes and improved glucose tolerance temporarily (11). Since that observation, numerous studies have confirmed the beneficial effects of exercise for patients with T2DM (12–17) and those at risk for diabetes, specifically those with insulin resistance (IR) and/or metabolic syndrome. There is strong evidence that these salutary effects include acute and long-term improvements in insulin sensitivity, changes in body composition, improvements in blood pressure (BP), endothelial function, and cardiopulmonary fitness, and weaker evidence for improvements in lipid profile, inflammatory state, oxidant load, mitochondrial function, and thrombotic potential. These benefits are reviewed in the first part of this chapter.

Paradoxically, despite extensive data indicating the importance of physical activity and exercise, 60 – 80% of adults with T2DM do not exercise sufficiently, and adherence to exercise programs is low in these patients (18, 19). While there may

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be some selection bias inherent in this observation, since sedentary people are more likely to develop diabetes, another possible contributor to this relationship is the observation that exercise performance is impaired in individuals with diabetes, even early in the disorder (20–23). Recent studies suggest that this exercise impairment also occurs in other insulin-resistant, diabetes-risk states such as polycystic ovarian syndrome (PCOS) and metabolic syndrome (24, 25). Thus a bidirectional, adverse relationship between sedentary lifestyle and IR may also occur in these prediabetic conditions. The data supporting this observation and possible mechanisms for decreased functional exercise capacity in diabetes are discussed in the remainder of the chapter.

Overall Benefits of Exercise in Insulin-Resistant States

Prevention of Diabetes

The role of exercise in the prevention of diabetes is unequivocal. Early epidemiological and sociological evidence demonstrated a strong inverse correlation between habitual physical activity and the incidence of diabetes. This evidence included the change in incidence of diabetes with the move away from a rural lifestyle that was observed in American versus Mexican Pima Indians (26). This epidemiological relationship between physical inactivity and diabetes risk has been observed across diverse populations including male college alumni, female college alumni, registered nurses, and British men (27). These observations were followed by a set of prospective studies, the Finnish Diabetes Prevention Study (28), the Da Qing Study (29), and the Diabetes Prevention Program (30). In all these studies, a diet and exercise intervention prevented transition from impaired glucose tolerance to diabetes in 50 – 60% of individuals. Only the Da Qing Study included an exercise-alone arm. The preventative effect of exercise in this arm was similar to that observed with diet alone and was independent of weight loss, though body composition was not addressed. Hamman et al. also found the beneficial effect of achieving the exercise goal on prevention of diabetes in the Diabetes Prevention Project, even among those persons who did not lose weight (31). The reasons for exercise being so successful in preventing diabetes are not completely known but likely include improved insulin sensitivity, decreased visceral adiposity even in the absence of weight loss, enhanced fatty acid utilization, increased mitochondrial function and/or content, and modulation of inflammation and oxidative stress.

Cardiovascular disease and all-cause mortality

The current literature, including meta-analyses covering over 2.6 million person-years of study, provides indisputable support for the reduction in cardiovascular disease (CVD) risk associated with physical activity and physical fitness (32).

A 2001 meta-analysis of 23 studies representing more than 1.3 million person-years of follow-up demonstrated a linear decrease in CVD risk with increased physical activity (7). The relationship to objective measures of physical fitness was more complex, with a precipitous decline in CVD risk occurring before the 25th fitness percentile (Fig. 15.1A). Overall, the benefits of fitness were greater than thoseof physical activity, with the most fit population achieving a two-thirds reduction in relative risk of CVD compared to the least fit population.

The protective effects of exercise are also seen in analyses of mortality. One study found that low CV fitness predicted cardiovascular and all-cause mortality in a cohort of 25,714 healthy men (3) (Fig. 15.1B). The same observation held true for a cohort of 1263 diabetic men (2), and the mortality benefit of CV fitness was observed even in obese subjects. The relationship among physical activity, obesity, and mortality has been addressed directly by Blair and colleagues (33). They examined subjects with body mass index (BMI) less than 25, 25–30, or greater than 30 and found that in all groups lower habitual physical activity was associated with increased mortality. Similar benefits and a similar dose–response have been demonstrated for people with diabetes (6, 8) (Fig. 15.1C), hypertensive men (34), smokers and nonsmokers, and individuals with elevated and normal cholesterol

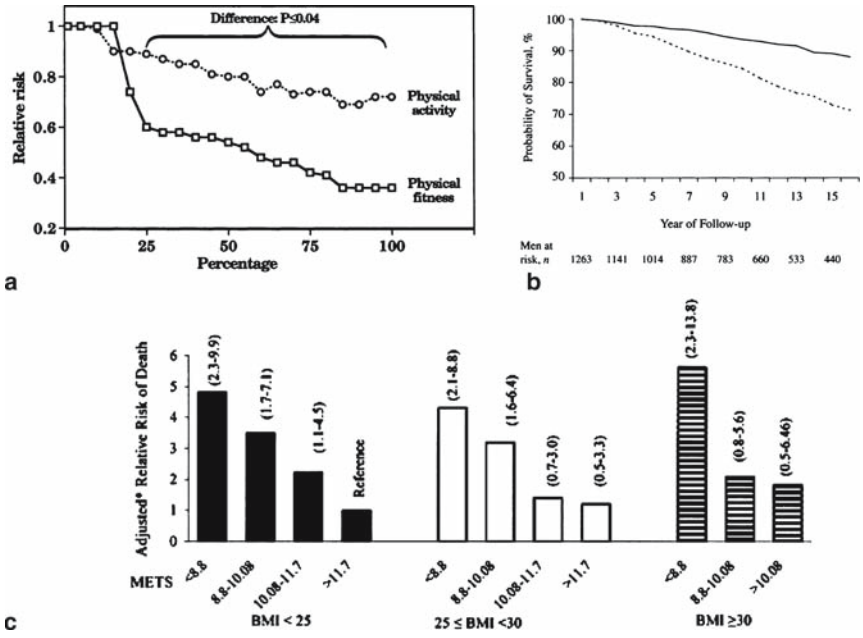

Fig. 15.1 (A) Estimated dose–response curve for the relative risk of CVD by fitness and physical activity (7); (B) Improved survival in cardiovascularly fit (solid line) vs. unfit (dotted line) men with T2DM over 12 years of follow-up in a cohort of 14,777 men (2); (C) Increased age-adjusted relative risk of all-cause mortality with decreased cardiovascular fitness in all weight categories in 2196 diabetic men over 32,162 person-years of observation (6). Reprinted from *Diabetes Care* and *Annals of Internal Medicine*

levels (35). In another epidemiological study, even occasional physical activity (one or less bouts per week) conferred a hazard ratio of 0.70 to 0.59 compared to no physical activity (5).

While this kind of evidence can be affected by selection bias and confounding variables, the consistency of the observations supports a cause and effect relationship between physical activity and decreased mortality that is biologically plausible, based on the impact of physical activity on lipids, blood pressure, endothelial function, carbohydrate tolerance, diabetes, and possibly inflammation and fibrinolysis.

It is well accepted that CVD risk and mortality are increased in individuals with diabetes and/or metabolic syndrome relative to those without these conditions and, as described earlier, that physical activity decreases CVD risk and mortality in both insulin-sensitive and insulin-resistant populations. An emerging trend in the literature suggests that physical activity may actually provide protection from the excess CVD risk in insulin-resistant states (3, 36). In other words, the most fit or active insulin-resistant individuals may have little or no increased CVD risk when compared to similarly fit insulin-sensitive individuals. In an analysis of leisure time activity and its ability to protect subjects from the excess CVD risk associated with insulin-resistant states, the Whitehall study found that increasing activity had a more pronounced protective effect in men with diabetes and impaired glucose tolerance (IGT) than in normoglycemic men (Fig. 15.2) (36). In fact, the most active

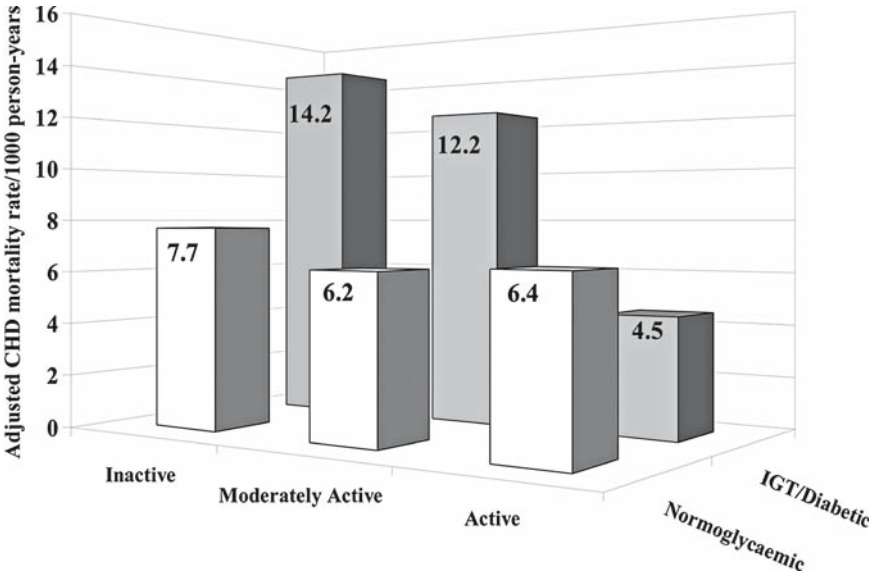

Fig. 15.2 Age-adjusted CVD mortality rates by leisure time activity in normoglycemic men (6056) vs. IGT/diabetic men (352) in the Whitehall Study (32) (Adapted by Gill and Malakova from data from the Whitehall Study (36)). $P = 0.006$ for trend in normoglycemic men, $P = 0.003$ for trend in men with IGT/diabetes

insulin-resistant group had an age-adjusted CVD mortality rate similar to that of the most active normoglycemic men. Furthermore, in the Aerobics Center Longitudinal Study, the increases in CVD and all-cause mortality associated with the metabolic syndrome and with obesity were eliminated or attenuated to less than statistical significance when mortality was adjusted for cardiorespiratory fitness, suggesting that the observed mortality effects of these conditions are largely explained by the lower cardiovascular fitness in these groups (4). Therefore, physical activity and/or cardiorespiratory fitness may have a particularly potent effect on mortality in insulin-resistant individuals, those with the highest baseline mortality rates.

The role of insulin sensitivity in the beneficial effects of physical activity on CVD risk has been directly addressed in a few studies that have adjusted for markers of insulin sensitivity (37). These studies suggest that improvement in insulin sensitivity is central to the CV-protective effects of exercise. For instance, in the Uppsala Longitudinal Study of Adult Men (ULSAM), adjustment for surrogate markers of insulin sensitivity, including fasting insulin and proinsulin, attenuated the risk of CVD mortality associated with an inactive life style (38). This is consistent with the findings of Bonora et al. in a 15-year prospective study of 839 new cases of symptomatic CVD (39). In this study, IR (by HOMA-IR) was an independent predictor of new CVD events (HR 2.2, 95% CI 1.4–3.6, $p < 0.001$) after adjustment for all traditional cardiac risk factors, as well as for the nontraditional risk factors of physical activity, BMI, triglycerides (TG) level, high sensitivity C-reactive protein (hsCRP), adiponectin, fibrinogen, vascular cell adhesion molecule-1 (VCAM-1), and oxidized low density lipoprotein (LDL). Therefore, it seems likely that IR itself (or the underlying cause thereof) increases CVD risk, and that exercise attenuates this increased risk by reversing IR.

Effects of Exercise on Glucose Tolerance and Insulin Sensitivity

Glucose metabolism in response to exercise has been extensively studied, as it poses an important clinical challenge. Exercise has two different impacts on carbohydrate metabolism: The bout effect and the training effect. The bout effect refers to the direct impact of a single episode of exercise on glucose disposal during the exercise and for an interval of 1–72 h after the exercise is complete. In contrast, exercise training is typically considered routine physical activity that increases functional exercise capacity, for which the gold standard is maximal exercise capacity reflected by the maximal oxygen consumption (VO_2max). In contrast to a single bout of exercise, exercise training usually also affects body composition, especially lean body mass, and metabolic flexibility (40). The overall benefits of regular exercise on insulin sensitivity likely result from a combination of bout and training effects.

Bout effects

It is well established that even a single bout of exercise has a pronounced effect on the metabolism of the person with T2DM. In fact, much of the metabolic benefit of training may be due to the most recent bout of exercise (41, 42). In support of the concept that single bouts of exercise affect metabolic parameters, Devlin and others reported that a single bout of glycogen-depleting exercise in patients with T2DM significantly increased glucose disposal for at least 12 to 16 h post-exercise, owing to an enhanced rate of nonoxidative glucose disposal (41). This increase occurs at the level of both liver and muscle tissue (43), probably peaks during or soon after exercise, and can persist for at least two days, but not five days (44, 45). Others have found that exercise conditioning for one week increases whole-body insulin-mediated glucose disposal (46) and glucose tolerance (12) in patients with T2DM. It is not completely clear how much metabolic benefit is derived from a single bout of exercise, as opposed to the effect of cumulative bouts, but it is clear that the benefit of a bout of exercise is lost rather quickly. Therefore, repeated exercise, probably daily or at least every other day, is needed for long-term bout-effect benefits on glucose metabolism. In addition, a very brief period of exercise, such as a single bout or even a week of exercise, is clearly insufficient to cause increases in maximal oxygen consumption, changes in body composition, or improvement in other CV parameters observed after a chronic training program, which have clear, independent mortality benefits, as well as potential independent effects on glucose metabolism.

Training Effects

The effects of exercise training or routine physical activity on insulin sensitivity and glucose tolerance are likely to be complex and multifactorial, and the relative roles of decreased visceral fat, CV fitness, and cumulative bout effects of exercise have yet to be defined (37). Recent studies clearly demonstrate that exercise training leading to increased fitness (generally defined as an increase in VO_2max) also results in improved insulin sensitivity, as measured by the gold standard hyperinsulinemic euglycemic clamp 72 h after the last bout of exercise (47, 48). These studies also compared exercise regimens consisting of moderate vs. high-intensity activity, but with equal exercise energy expenditure, and found greater effects on insulin sensitivity with higher-intensity physical activity despite similar effects on VO_2max. These results suggest that fitness alone may not be the primary determinant of insulin sensitivity, although there is clearly a relationship.

Others have asked whether the benefits of long-term exercise training (as opposed to the bout effect) on insulin sensitivity can be completely accounted for by changes in visceral adiposity. These studies have had mixed results (37). For instance, Christou et al. performed a cross-sectional analysis of 135 men aged 20–79 and found that

both low fatness and high fitness correlated with insulin sensitivity. However, in multiple regression analyses adjusting for strong measures of total or visceral adiposity (waist circumference and total body fat), VO_2max was not an independent predictor of insulin sensitivity or fasting insulin concentration. In contrast, after adjustment for fitness (VO_2max), fatness remained an independent predictor of both measures of insulin responsiveness (49). These data suggest that insulin sensitivity is more tightly associated with low visceral adiposity than with high fitness. Similarly, in another cross-sectional study of 407 adults, multivariate analyses estimated that VO_2max, though an independent predictor of insulin sensitivity, explained only about 1 – 2% of the variance, whereas waist circumference explained approximately 20% of the variance (50), again suggesting a much more significant effect from fatness than from fitness. However, Lee et al. examined overall metabolic syndrome risk, rather than insulin sensitivity alone, in 297 adult men. They found a relative risk for metabolic syndrome in the lowest fitness quintile vs. the highest fitness quintile of 4.6 and 1.8, respectively, before and after adjustment for abdominal fat (visceral and subcutaneous). Therefore, when considering the overall metabolic risk, rather than IR alone, adiposity and fitness both appear to play important roles (51).

In general, evidence suggests that the benefits of physical activity on overall metabolic risk result from a combination of single and cumulative bout effects on insulin sensitivity/glucose tolerance, training effects (through fitness and body composition) on insulin sensitivity and other metabolic syndrome criteria, as well as effects (described below) on inflammation, endothelial function, and hemostasis.

Mechanisms of improved glucose tolerance with exercise

Introduction

The mechanisms by which exercise increases insulin sensitivity and glucose tolerance are complex and multifactorial. Several mechanisms that are likely to be involved have been proposed and supported in the literature (32, 37). These mechanisms are discussed in an approximate progression from acute to more long-term effects.

Immediate effects on glucose utilization

Numerous studies have demonstrated direct benefits of exercise on measures of glucose utilization. For instance, Perseghin et al. found increases in human skeletal muscle glycogen synthesis and glucose transport and phosphorylation

with exercise (45). They studied both a single bout of exercise and a 6 week training regimen and found that most of the increase in both parameters (about 60%) was present 48 h after the first single bout of exercise. These increases occurred both in insulin-sensitive individuals and in insulin-resistant offspring of diabetic subjects. They do not comment on weight or body composition changes after the 6 weeks of training. However, exercise intensity was increased to maintain a fixed heart rate response suggesting that fitness did improve. Therefore, the additional benefit after training may represent a training effect or a cumulative bout effect.

The increase in muscle glucose utilization is a combination of insulin-independent and insulin-dependent effects. The immediate response to acute exercise is a muscle-contraction-induced, insulin-independent translocation of the glucose transporter, GLUT4, to the cell surface. This appears to be mediated by two signaling events (52). One signal, probably the first, comes from release of calcium from the sarcoplasmic reticulum during muscle contraction and acts via activation of calcium/calmodulin-dependent protein kinase (CaMK) II. In the other signaling event, the increase in the adenosine monophosphate to adenosine triphosphate ratio (AMP:ATP) that occurs with contraction activates AMP-activated protein kinase (AMPK) via phosphorylation, primarily by LKB1. Use of inhibitors to block either of these pathways in rodent skeletal muscle blocks acute contraction-mediated increases in glucose transport. The significant downstream events in these pathways that lead to increased muscle Glut4 activity are not understood.

Later in a bout of exercise, this insulin-independent enhanced glucose utilization appears to be supplemented or replaced by an increase in skeletal muscle insulin sensitivity, though there is controversy as to whether this occurs through increased insulin signaling or through some other mechanism. Some studies have found evidence for increased tyrosine phosphorylation of insulin receptor and insulin receptor substrates 1 and 2 (IRS1 and 2), as well as increased PI3 kinase binding to IRS and PI3 kinase activity following a bout of exercise (53). This increased signaling would presumably result in the maintenance of increased skeletal muscle GLUT4 activity, as well as transcriptional changes induced by insulin action. However, others have found that this post-exercise insulin sensitivity does not involve increases in insulin signaling per se or require new protein synthesis (52, 54). In contrast, they find that insulin sensitization does occur, as evidenced by a left shift of the insulin dose curve, but with concomitant lowering of serum insulin levels and no increase in direct measures of insulin signaling. In these studies, exercise-induced insulin sensitization is dependent on a serum factor (possibly interleukin (IL)-6) and the effect of exercise is mimicked by hypoxia and by activators of AMPK (52, 55) (see below).

Overall, it appears that a bout of exercise induces immediate insulin-independent increases in glucose uptake (minutes to hours), followed by enhanced insulin action, with increased insulin-dependent glucose transport and glycogen synthesis (hours to days). There is evidence to suggest that the exact duration of increased insulin sensitivity following a bout of exercise depends on the rate at which glycogen stores are depleted and, therefore, on the intensity and duration of exercise as well as on post-exercise diet (52).

Inflammation

Inflammation is one of the universal mechanisms contributing to the initiation and progression of atherosclerosis (56) and the development of T2DM (57, 58). It is also thought to play a role in the development of IR in these conditions. Infusion of tumor necrosis factor-α (TNF-α) has been shown to induce skeletal muscle IR via inhibition of Akt pathways (59). Therefore, anti-inflammatory effects of exercise may contribute to its insulin-sensitizing effects. However, the inflammatory consequences of exercise remain unclear.

In general, short-term, moderate-intensity exercise interventions have a modest positive impact on some subset of circulating cytokines, such as IL-1 and -18, CRP, and TNF-α; presumed anti-inflammatory markers such as adiponectin (and IL-6, see below); and inflammation-related cell adhesion molecules such as VCAM, ICAM, and the selectins (60–65), but exact methods and results have varied. For instance, Zoppini et al. found stable CRP and decreased ICAM and P-selectin following 6 months of aerobic exercise in older, sedentary, overweight subjects with diabetes (61). In contrast, Olson et al. found reduced CRP and increased adiponectin, but stable cell adhesion markers after 1 year of resistance training in overweight women (60). In addition, there are studies that do not demonstrate any exercise-induced change in circulating inflammatory markers (66). One of the largest studies to address this issue is the health, risk factors, exercise training and genetics (HERITAGE) Family Study of 652 healthy sedentary adults (67). A 20-week exercise intervention failed to reduce CRP in the whole study population, but did reduce CRP by about 25% in the subpopulation with a baseline CRP >3 mg/l. Thus, evidence suggests that the effect of exercise on inflammation is dependent upon the baseline status of the population and on the nature, intensity, and regularity of the exercise intervention. It is likely that a muscle-damaging level of exercise can cause inflammation, while more modest or habitual exercise reduces systemic inflammation to some degree.

Another proposed mechanism for variability in exercise inflammatory response invokes oxidant stress. Increased metabolic rate in the exercising muscle results in increased generation of oxidants that can cause tissue damage. With training, levels of antioxidant enzyme systems increase and oxidant damage decreases (68). The net result is, therefore, a balance between two opposing forces: inflammation and oxidant stress from unaccustomed activity, and anti-inflammatory, antioxidant responses to habitual or modest activity. Further studies are clearly necessary for a better understanding of the effects of exercise on systemic inflammation.

A further complication arises from the fact that one of the presumed proinflammatory cytokines that is frequently measured in studies of inflammation in metabolic syndrome, obesity, and diabetes is IL-6. A large body of recent data suggests that IL-6 has pleiotropic effects that include significant metabolic and insulin sensitizing effects, as well as possible anti-inflammatory effects (69, 70). IL-6 is produced by skeletal muscle during sustained exercise, and plasma levels of IL-6 are transiently elevated dramatically (on the order of 100-fold) in response to exercise. Infusion of recombinant IL-6 has been shown to inhibit production of TNF-α in

response to endotoxin. Other evidence supports a role for IL-6 in suppression of TNF-α production and in the synthesis or release of other anti-inflammatory molecules such as IL-1RA (IL-1 receptor antagonist) and soluble TNF- α receptor (65). Furthermore, studies of IL-6-deficient mice have demonstrated that these animals have decreased exercise endurance, decreased O_2 consumption during exercise, and impaired fatty acid oxidation in response to exercise (71). These effects appear to be mediated by a decrease in induction of AMPK activity and of fatty acid oxidation pathways in exercising muscle, by decreased lipolysis in adipocytes and glucose release from the liver, and by a decrease in sympathetic outflow during exercise (69). Overall, the literature is consistent with a crucial role for IL-6 in exercise performance and in the generation of a high-turnover metabolic state during exercise and other forms of physical stress. Interestingly, by 9 months of age, IL-6 deficient mice are obese and have several features of metabolic syndrome, including IGT. Clearly, a full understanding of IL-6's complex role in exercise, metabolism, diabetes, and inflammation awaits further study.

Mitochondrial function and fatty acid oxidation

Another mechanism by which exercise may improve insulin sensitivity is through its effect on mitochondrial function and muscle oxidative capacity. There is ample evidence that a high level of fatty acids or TG in the serum can cause IR. Similarly, high intramuscular TG (IMTG) levels are associated with IR in patients with diabetes and other insulin-resistant populations. Paradoxically, athletes also have high intramuscular TG levels, but are exquisitely insulin sensitive. It has been hypothesized that this case, where fat is actively accumulated to provide fuel for an accustomed high level of activity, is different from the diabetic muscle, in which fat accumulates because of an abundant supply that is not efficiently metabolized (72). Increasing evidence suggests that a mismatch between fat supplies and fat oxidation leading to accumulation of fatty acids, TG, and their more reactive intermediates contributes to IR. Fatty acids, diacylglycerol, ceramides, and long-chain acyl-CoA can all inhibit insulin signaling by activation of protein kinase C (PKC), with subsequent inhibitory serine phosphorylation of insulin receptor substrates (IRS) (73).

The underlying defect is thought to be a deficiency in skeletal muscle oxidative capacity, especially for fatty acid oxidation. Activities of muscle mitochondrial oxidative enzymes have been found to be robust predictors of insulin sensitivity in human subjects (74). A number of recent studies have demonstrated decreased mitochondrial content and/or function in insulin-resistant individuals (75, 76). For instance, Peterson et al. compared lean, healthy offspring of type 2 diabetic parents to lean, healthy controls and found marked IR in the offspring. This was associated with increased IMTG despite similar levels of lipolysis and with significantly lower levels of mitochondrial ATP synthesis (77). Furthermore, the level of carnitine palmityltransferase 1 (CPT1), the rate-limiting step for mitochondrial uptake of fatty acids for oxidation, is reduced in insulin-resistant muscle (78, 79). Therefore,

it is likely that skeletal muscle IR is associated with, and potentially caused by, fatty acid supply in excess of the tissue's ability to oxidize or safely store fatty acids.

How does physical activity affect this imbalance in fat supply over fat utilization? Goodpaster et al. studied the effects of training combined with caloric reduction on insulin sensitivity and fat oxidation. They found that fasting fat oxidation increased by nearly 20% after the intervention and that it was a stronger predictor of insulin sensitivity than weight loss or fitness (80). In another study by the same group, a similar intervention was found to induce about 50% increase in skeletal muscle mitochondrial electron transport chain activity (81, 82). Unfortunately, these studies do not allow a distinction between the effects of training and those of negative energy balance, both of which promote increased insulin sensitivity. The authors do, however, note that in a previous study using caloric restriction without exercise, a smaller improvement in insulin sensitivity was achieved and no increase in fat oxidation was detected (83). Furthermore, in another study, a physical activity intervention was implemented in a sedentary, overweight to obese population without caloric restriction (84). Fat oxidation and insulin sensitivity were measured after 2–4 bouts of exercise (less than 1week of training) and after 6 weeks of training. Significant and maximal increases in insulin sensitivity and fat oxidation were present after 2–4 bouts of exercise in the absence of changes in weight or fat distribution.

The implication that improved fat oxidation and mitochondrial function are a bout effect of exercise is supported by a body of literature. For instance, Holloway et al. demonstrated that during 120 min of aerobic exercise, whole-body fat oxidation, mitochondrial palmitate oxidation, and CPT1 activity increased progressively throughout the exercise bout (85). Furthermore, a recent study demonstrated that a single bout of exercise is able to prevent the IR induced by lipid/heparin infusion (86). Compared to a sedentary control day, the exercise bout increased enzymes of TG synthesis, decreased muscle diacylglycerol levels, and increased whole-body fat oxidation throughout the lipid infusion. Like the bout effect of exercise on glucose disposal, this effect is mediated at least in part by the activation of AMPK by muscle contraction (87, 88). Thus, one apparent bout effect of exercise that contributes to insulin sensitization is an increase in the ability of muscle to oxidize fat through increases in mitochondrial activity.

AMPK

AMPK has been mentioned in previous sections on glucose utilization, inflammation, and fatty acid oxidation. However, it merits its own summary section, as it is likely a key mediator of many of the beneficial effects of exercise (87, 88). The human AMPK gene is a homolog of a key regulatory gene in yeast, SNF1, that controls the transition from glucose fermentation to utilization/oxidation of other fuel sources. The human gene appears to have a similar role in promoting non-glucose fuel utilization. However, recent studies have also demonstrated that AMPK increases glucose uptake and inactivates glycogen synthase (or limits its activation

during glycogen depletion), thereby also increasing glucose fuel availability. Thus, AMPK acts as a cellular fuel gauge and general promoter of fuel supply and utilization during high energy flux states, such as exercise (88). AMPK is also activated by hypoxia and other low ATP conditions that are interpreted as low fuel availability states, as well as by analogs of AMP.

Overall, AMPK has been shown to have multiple fuel-promoting effects in skeletal muscle, including insulin-independent translocation of GLUT4 to the cell surface, inhibition of glycogen synthase, inhibition of fatty acid synthesis through inhibition of acyl CoA carboxylase, indirect activation of fatty acid transport into mitochondria by CPT1, induction of expression of GLUT4 and of multiple genes involved in fatty acid oxidation, induction of PGC-1α (peroxisome proliferatory-activated receptor-γ coactivator-1α, an enzyme involved in mitochondrial biogenesis), and activation of endothelial nitric oxide (NO) synthase, leading to improved vasodilatory responses (88). AMPK also inhibits insulin secretion, contributing to the metabolic shift from fuel storage to fuel utilization. Increased sensitivity to insulin at the level of skeletal muscle still allows efficient glucose uptake, but AMPK-mediated inhibition of glycogen synthase then directs this glucose to utilization rather than storage (89).

AMPK is activated by muscle contraction either during exercise or by direct electrical stimulation. Interestingly, muscle contraction also increases AMPK activity in liver and other tissues, promoting concomitant adaptations to exercise in non-muscle tissues. AMPK activity increases immediately after muscle contraction and remains elevated for about 30 min after cessation of exercise. The degree of activation is highly dependent on the amount of mobilizable muscle glycogen and on the intensity of exercise. AMPK activation also appears to play a role in training effects of exercise. With regular exercise, repeated activation of AMPK and repeated induction of genes involved in mitochondrial synthesis and function lead to increases in mitochondrial content and activity (probably through PGC-1α) and, therefore, in muscle oxidative capacity and VO_2max. It is also possible that repeated activation of AMPK has chronic effects on the vasculature, improving endothelial function and microvascular blood flow. Interestingly, metformin, the oral insulin-sensitizing agent demonstrated to prevent diabetes to a significant degree, has also been shown to activate AMPK. It is possible that this activation is the mechanism by which metformin exerts its insulin-sensitizing effects.

Much remains to be understood about this central regulator of exercise-induced changes in metabolism, but this unfolding story of interplay between AMPK, insulin, and glycogen promises to explain much of demand-regulated metabolic adjustment during exercise.

Endothelial function

Since glucose uptake by tissues is dependent on delivery of glucose to the tissues, changes in endothelial function may also affect glucose disposal. Changes in muscle blood flow and, specifically, in blood flow distribution mediated by the endothelium,

increase delivery of glucose to tissues that are most metabolically active. It is possible that endothelial dysfunction in diabetes contributes to IR by impairing appropriate blood flow distribution. Conversely, improvements in endothelial responsiveness may contribute to improved glucose homeostasis in response to regular physical activity.

Exercise training improves endothelial function in the context of metabolic and CV disease. For instance, in a study of patients with congestive heart failure (CHF), 4 weeks of lower-leg exercise training significantly improved upper-extremity endothelium-dependent vasodilation (90). Another study demonstrated improved flow-mediated dilatation (FMD) with a 12-week treadmill training program in hypertensive men (91). Such responses to exercise training have also been demonstrated in subjects with insulin resistance and diabetes. Kelly et al. showed that 8 weeks of stationary bike training significantly improved brachial artery FMD in a group of overweight children relative to a sedentary control group (92). Similarly, Meyer et al. found improved endothelial function after 6 months of endurance training in obese, sedentary children (93). Two recent studies demonstrated improvement in endothelium-dependent vascular reactivity after 8 weeks of exercise training in overweight and T2DM adult subjects (94, 95). A third study showed improvement in biomarkers of endothelial function after 6 months of training in older patients with T2DM (61). Although most studies have investigated long-term exercise effects, one study demonstrated improved fasting and postprandial vascular function after a single bout of moderate exercise (96). In contrast, in a study of T2DM subjects, no improvement was seen in microvascular function, as measured by maximum skin hyperemia, after 6 months of aerobic exercise training (97).

Other studies have failed to find benefits of exercise on endothelial function in healthy individuals without baseline endothelial dysfunction, for instance in healthy relatives of T2DM individuals (62) and in healthy middle-aged men (98). The weight of evidence suggests that exercise training does significantly reverse impairment in endothelial function, but has no significant impact on normal vessels. The mechanism of restoration of endothelial function by exercise is poorly understood, but may involve increases in endothelial NO synthase (possibly via AMPK as described earlier) or increases in precursors to or function of NO.

Weight loss/maintenance

The worsening epidemic of obesity is clearly an underlying factor in the increasing prevalence and earlier onset of metabolic syndrome and T2DM. Exercise conditioning may serve as an adjunct therapy to aid in maintenance of weight loss, especially when linked to dietary change. However, in the absence of diet, exercise does not consistently lead to weight loss. In fact, exercise training without dietary change typically results in minimal absolute weight loss despite greater than 60–90 min a day of moderate activity (99). Although weight loss may not occur with exercise alone, body composition may be improved (100). These changes in body

composition include decreased visceral adiposity, and therefore may have significant beneficial effects on insulin sensitivity, as well as on other metabolic syndrome criteria and CV risk factors.

In contrast to the limited impact of isolated exercise for weight loss per se, exercise is very effective for acceleration of weight loss in combination with diet and, most importantly, maintenance of weight loss. In a community-based study, introduction of walking in combination with healthy snacks prevented weight gain (101). Similarly, in the National Weight Control Registry, comparison of a group of subjects who have maintained a substantial weight loss for more than 12 months with those who regained weight suggests that physical activity level of greater than 2000 calories per week is a crucial element of long-term success (102). When exercise is involved in a weight loss program, similar results have been reported. For example, a caloric restriction intervention resulted in a weight loss of 10 kg in the diet arm and 14 kg in the diet plus exercise arm. After 12 weeks, the dietary intervention was discontinued but the exercise intervention continued. At 36 weeks, the diet group had regained all but 4 kg, whereas the exercise group maintained 12 kg of weight loss (103). It is critical to convey to patients that exercise alone does not lead to weight loss so that they will have an appreciation of the role of exercise and not be discouraged by an apparent lack of weight-loss results from their exercise regimen.

Other beneficial effects of exercise

Lipids

The role of exercise interventions in altering lipid levels remains unclear (104). In general, studies with longer interventions (>6 months) of higher intensity and longer bout duration have shown increases in high density lipoprotein cholesterol (HDLC) levels and reductions in cholesterol and TG levels. For instance, in 111 sedentary, overweight men and women with mild to moderate dyslipidemia using a range of exercise intensities to carry out exercise training, Kraus et al. found significant reduction in LDL cholesterol (LDLC) and TG levels and improvement in HDLC level, with the highest intensity intervention and increases in LDLC particle size in all exercise groups after 6 months (105). However, a recent study with a 9 month running intervention in young healthy adults showed only a non-significant trend towards LDLC lowering and no effect on HDLC or TG levels despite a 24% increase in peak VO_2 (104). Baseline HDLC level was quite high in this study (mean of 63 for both groups), which might have limited the benefit. However, a significant reduction in apolipoprotein B (apoB) was reported, suggesting again that exercise might induce anti-atherogenic changes in LDLC particle size.

Two recent meta-analyses also found conflicting results with regard to the HDLC-lowering effects of exercise. A meta-analysis of studies of 2–12 months of

exercise in subjects with T2DM found a significant decrease in TG levels, but no significant change in HDLC or LDLC levels (106). In contrast, a recent meta-analysis of randomized controlled trials of exercise intervention with lipid end points (107) found a modest, but statistically and clinically significant, increase in HDLC (2.53 mg/dl, $p < 0.001$) with aerobic exercise training. A minimum of two hours per week of exercise was required, and univariate regression analysis suggested that the most important single factor was exercise duration per session. In addition, the greatest benefit was seen in individuals with higher total cholesterol levels and lower BMI at baseline. Unfortunately, the effects of diet have not been distinguished from those of exercise in most available studies, so the relative roles of each are unclear with regard to lipid effects.

Overall, HDLC response to exercise training is variable and appears to depend upon multiple factors including dose, time from last bout of exercise, gender, body composition, baseline lipids, and genetic background. As summarized by Ring-Dimitriou, studies support the need for longer-duration, higher-intensity exercise for significant effects on HDLC level (104). They also suggest that gender differences may exist, with greater benefit occurring in men than women. This may, at least in part, reflect higher baseline HDLC levels in premenopausal female subjects. It is possible that as HDLC levels fall with low estrogen in postmenopausal women, the effect of exercise on HDLC levels more closely resembles that seen in men, but this has not been studied. Recently, it was reported that there may also be genetic determinants of the HDLC cholesterol level response to exercise. A polymorphism in the PPAR-δreceptor (more common in Caucasians) was associated with a significantly greater improvement in HDLC with exercise training (108).

It has been proposed that exercise exerts its effects on lipid levels and lipid particle sizes largely through acute effects on TG levels mediated by increased TG clearance in exercising skeletal muscle, especially when glycogen-depleted (32). This is consistent with many of the observations above, as lipid effects would then require long-duration exercise, would be due to a bout effect and independent of overall fitness, and could be affected by polymorphisms of PPAR-δ, a nuclear receptor involved in regulation of fatty acid oxidation. Overall, it is reasonable to conclude that exercise and/or exercise training may have a positive effect on lipids (HDLC raising, TG lowering, and possibly LDL particle size effects), but it should not be employed in lieu of lipid-lowering pharmacotherapy when indicated. At present, it does represent one of the very few interventions, and arguably the safest intervention, with potential for raising the HDLC level.

Fibrinolysis

In addition to the well-established risk factors, an elevated level of plasma fibrinogen has also been reported to be a CV risk factor. Acute, exhaustive exercise stimulates both thrombosis and fibrinolysis, with a net neutral effect on hemostasis in most populations (109, 110). In the general population, the chronic and immediate

post-exercise responses in the thrombotic and fibrinolytic systems have been shown to be variable and reflect differing adaptations with aging and responses to various exercise protocols. In a recent study, investigators examined hemostatic variables, including factor VII activity (FVIIa), tissue factor pathway inhibitor-factor Xa complex (TFPI/Xa), and plasminogen activator inhibitor-1 (PAI-1) antigen/activity, after a high fat meal before and after exercise training (111). They observed reduction in the potential for coagulation and improved fibrinolytic potential in trained subjects after the meal stimulation, suggesting that under certain conditions (e.g., postprandially) exercise may have beneficial effects on hemostasis. Though the mechanism is not understood, this effect may be related to the anti-inflammatory effects of exercise.

In people with diabetes, results are similarly variable. Fibrinogen level is elevated in men and women with T2DM (112), but it is not clear whether exercise training decreases fibrinogen level in T2DM. Schneider et al. found that although VO_2max increased by 8% with 6 weeks of training, fibrinogen level did not change significantly in a group of sedentary persons with T2DM (113). Conversely, Hornsby et al. found that a 12.5% increase in VO_2max after 12–14 weeks of training was associated with a significant decrease in fibrinogen level in sedentary persons with T2DM (114). In the Finnish Diabetes Prevention Study, a combined diet and exercise intervention decreased PAI-1 level, consistent with improved fibrinolysis (115). Another recent study found improved fibrinolysis after 6 months of aerobic training in overweight to obese men and women (116). Interestingly, improvements were significantly greater in men than women and correlated closely with the degree of abdominal fat. Therefore, the effect of exercise training on fibrinolysis appears to be generally salutary and may be mediated by changes in body composition, but this relationship requires further investigation.

Blood pressure

High BP is a leading contributor to CV mortality, and there is a consistent inverse relationship between physical activity and BP in cross-sectional studies. The first study to examine the impact of training upon BP was conducted by Jennings with a very rigorous exercise program in sedentary men (117). Over the last few decades, a dose–response effect of exercise on BP has been observed in both men and women, including those with CV and metabolic co-morbidities.

A recent meta-analysis assessed 72 longitudinal intervention studies to determine the impact of exercise training on BP (118). Studies included both hypertensive and normotensive subjects. Overall, the analysis demonstrated a small (3 mmHg), but clinically and statistically significant, decline in both systolic and diastolic average BP at rest, with a greater reduction in hypertensive subjects. The authors concluded that endurance training decreases BP through a reduction in systemic vascular resistance secondary to decreased sympathetic nervous system and renin–angiotensin system activity. In a recent study of 30 obese T2DM subjects, a

three-month exercise intervention improved both systolic and diastolic BP (119). Overall, improvement of BP with exercise training is the most consistently demonstrated benefit of routine physical activity on traditional CV risk factors.

Effects of T2DM on Exercise Performance

Introduction

Persons with metabolic syndrome or T2DM are at higher risk for coronary artery disease, stroke, and peripheral arterial disease due to accelerated atherosclerosis than individuals without these conditions (120). As discussed earlier, exercise appears to be especially beneficial in these individuals and may actually eliminate the excess risk of CVD seen in these conditions (42, 121). Furthermore, the observed beneficial effects of physical activity on insulin sensitivity and glucose metabolism make it clear that, in addition to reducing CVD morbidity and mortality, exercise training, or even an increase in the level of habitual physical activity, has a key role in the management of insulin-resistant conditions. Yet, population studies indicate that people with T2DM are generally less active than non-diabetic people (18). While some aspects of this behavior may be accounted for by lifestyle choices that contribute to the initial development of diabetes, recent evidence suggests that pathophysiological factors may also contribute to this decrease in activity. This section will discuss defects in CV or cardiopulmonary exercise performance observed in subjects with T2DM or IR syndromes and explore possible mechanisms for these defects.

Maximal exercise capacity

Studies have clearly demonstrated that people with T2DM, even very early apparently uncomplicated diabetes, have reduced CV exercise performance compared with non-diabetic persons matched for age, weight, and/or physical activity as evidenced by a lower VO_2max during incremental exercise (e.g., Table 15.1) (15, 16, 22, 122–125). The overall difference in VO_2max between healthy persons and persons with T2DM is approximately 20%. The mechanisms for this impairment have not been completely elucidated. However, on the basis of available data, central cardiac and peripheral factors limiting systemic oxygen delivery, as well as defects in tissue oxygen extraction, may all play a role (see below).

Many reports have suggested that the reduced VO_2max in T2DM correlates with IR (126–128). IR has also been reported to be inversely correlated with VO_2max in several disease states in addition to diabetes, including heart failure and chronic renal failure (129, 130). That this decrease in exercise capacity is independent of

Table 15.1 Maximal Exercise Capacity

	Lean Control	Obese Control	DM
Age (years)	36 ± 6	37 ± 6	42 ± 7
Fat-free mass (kg)	42 ± 7	48 ± 5	47 ± 5
HgbA1c (%)	6.0 ± 0.6	5.3 ± 0.5	9.0 ± 0.4*
BMI (kg/m^2)	23.5 ± 2.3†	30.8 ± 3.6	33.1 ± 6.3
Maximal exercise response			
VO_2max (ml/kg/min)	25.7 ± 4.9	22.0 ± 2.3	17.1 ± 3.8*
Maximal RER	1.13 ± 0.05	1.13 ± 0.08	1.16 ± 0.11

RER, respiratory exchange ratio; *P <0.05 for difference between T2DM and controls. †P <0.05 for difference between lean and other two groups. Data are mean ± SD. (Printed with permission from *J. Applied Physiol* (22, 125))

other complications of diabetes or of the systemic illness associated with heart and renal failure is supported by the recent findings of exercise defects in non-diabetic women with PCOS (24) and in metabolic syndrome (25). The significant decline in VO_2max in subjects with PCOS compared to age- and weight-matched controls with similar physical activity levels correlated with all measures of IR, but not with BP, cholesterol, or androgen levels. In addition, an association between IR and low physical fitness level has been demonstrated in normotensive men with a family history of hypertension (131).

The cause and effect relationship between IR and impaired exercise performance is not well understood and has been further addressed through the use of a pharmacological intervention to improve insulin sensitivity. In a study of 20 women with early, uncomplicated T2DM randomized to rosiglitazone or placebo, rosiglitazone treatment resulted in a significant improvement in VO_2max of 7% (126). This improvement correlated with both increased insulin sensitivity and improved endothelial function. The implication of this result is that the exercise impairment associated with IR is not solely a result of deconditioning from an associated sedentary lifestyle, but may actually be a direct result of IR. Therefore, the above data suggest that the cause and effect relationship between low physical activity and diabetes may be bidirectional. Not only does lack of exercise promote IR, but IR appears to cause defects in functional exercise capacity. Again, this relationship appears to exist outside the context of diabetes and to relate to IR in a variety of different settings.

Submaximal Exercise Tolerance and Oxygen Uptake Kinetics (VO_2 kinetics)

In T2DM, the exercise abnormality observed at maximal exercise is also observed during less vigorous physical activity (i.e., submaximal exercise). During the early stages of an incremental exercise test, oxygen uptake (VO_2) increases with each

increase in work rate. In non-diabetic individuals, there is a predictable increase in VO_2 to meet the metabolic demand for a given increase in workload (e.g., ~10.1 ml/min/W) (132). The VO_2-to-work load relationship thus describes an individual's overall ability to adjust to the exercise stress, and reductions in the slope of this relationship have been shown to effectively indicate abnormalities of cardiac output and gas exchange in cardiopulmonary and vascular diseases (133).

Similar to maximal O_2 consumption, the increase in VO_2 per unit increase in workload is reduced in people with T2DM compared to healthy controls (22). Potential mechanisms for this abnormal response include a decrease in oxygen delivery due to impaired endothelial or cardiac function and/or an abnormality of muscle oxidative metabolism. To further evaluate these possibilities, submaximal constant-load exercise has been employed. Unlike graded or incremental exercise, constant-load exercise is performed at a moderate workload below the individual's lactate threshold, where a steady-state VO_2 for a given work rate can be obtained. Following the onset of exercise, VO_2 rises exponentially to a new steady state. The time course of this rise, representing the VO_2 kinetic response, is determined by the systemic integration of muscle VO_2, CV adaptations of oxygen delivery, and pulmonary gas exchange. Three phases of the pulmonary VO_2 response to the change from rest to moderate constant-load exercise have been proposed (134, 135). At the onset of exercise, pulmonary VO_2 in the lungs increases abruptly for the first 15–20 s as cardiac output and pulmonary blood flow initially increase (cardiodynamic phase or phase 1). Following a circulatory transit delay (usually about 20–40 s), VO_2 then increases exponentially (phase 2), reflecting the increase of muscle VO_2 as tissue oxygen extraction and blood flow increase to meet the exercise demand (136, 137). This is the primary component of VO_2 kinetics and is described by a time constant (τ_2) reflecting the time to reach ~63% of the increase in VO_2. Phase 2 ends as muscle VO_2 and pulmonary gas exchange reach a steady state or phase 3. VO_2 kinetics may be limited by defects in oxygen delivery to the working tissues or by the inertia of oxidative metabolism in the working tissue (136, 138). Thus the time constant (τ_2) of phase 2 VO_2 kinetics is sensitive to alterations in oxygen exchange at the lungs, cardiac output, endothelial function, oxygen diffusion, and rates of tissue oxygen consumption and is prolonged in patient groups with abnormal pulmonary, CV, or metabolic responses to exercise.

We have observed that the VO_2 kinetic response is slowed in women with T2DM compared to non-diabetic women of similar BMI and physical activity levels in the absence of any clinical evidence of CVD (22) (Table 15.2). We assessed VO_2max (see Table 15.1 above), submax VO_2, VO_2 kinetic responses, and heart rate kinetic responses (measuring rate of rise of heart rate at the beginning of exercise). Women with T2DM had not only a lower VO_2max but also reduced VO_2 at all submaximal work loads (Fig. 15.3) and slower VO_2 and heart rate kinetic responses than either obese or lean non-diabetic controls (Table 15.2). These data suggest that diabetes, rather than obesity per se, is responsible for the observed exercise impairments. However, the magnitude of the defect correlates with fasting insulin, again suggesting that IR is a key determinant of the exercise impairment. In addition, the finding that heart rate kinetics is slowed in diabetes suggests a cardiac or "central" oxygen delivery component to exercise impairment (22).

Table 15.2 Submaximal Exercise Kinetics

	LC	OC	DM
VO_2 kinetics			
20 W τ (s)	21.4 ± 8.9	18.4 ± 9.9	42.6 ± 23.8*
30 W τ (s)	28.8 ± 5.3	27.8 ± 8.9	36.8 ± 6.2*
80 W τ (s)	42.8 ± 7.5	41.2 ± 8.2	55.7 ± 20.6
Heart rate kinetics			
20 W τ (s)	8.5 ± 4.6	10.6 ± 8.2	23.8 ± 16.2*
30 W τ (s)	23.9 ± 13.8	14.2 ± 8.0	40.7 ± 11.9*
80 W τ (s)	41.2 ± 14.8	43.3 ± 11.3	72.3 ± 21.5*

LC, lean controls; OC overweight controls; DM, T2 diabetes; W, watts; τ, the monoexponential time constant of VO_2; *: P <0.05 difference between T2DM and both control groups. Data are mean ± SD. (Printed with permission from *J Applied Physiol*. (22))

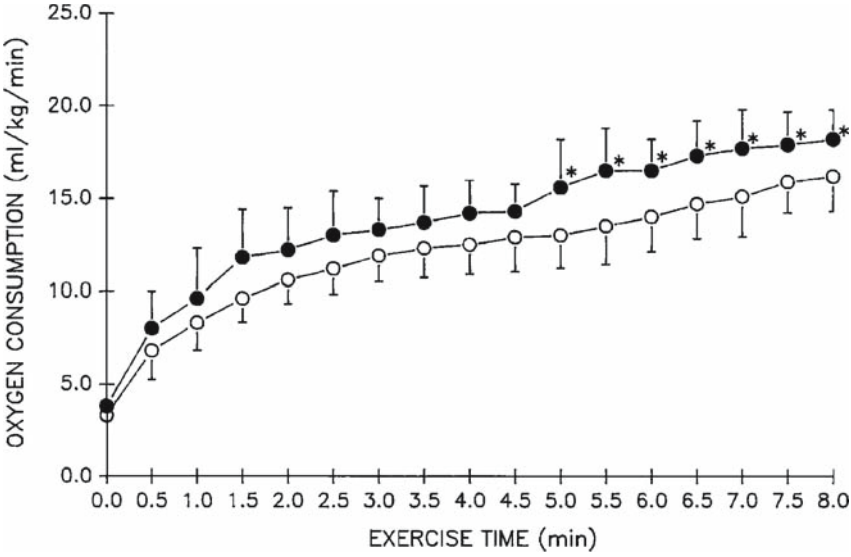

Fig. 15.3 Oxygen consumption in type 2 DM. This figure illustrates that oxygen consumption at all submaximal work loads for which there is complete data is reduced in persons with type 2 DM (open circles) compared to nondiabetic controls (closed circles) of similar age and activity levels during graded exercise testing (23). Reprinted from *Med Sci Sports Exerc*

Potential mechanisms for IR-associated exercise impairment

While the positive effects of exercise on insulin sensitivity are clear, the above results support the hypothesis that IR, in turn, negatively affects exercise capacity. There are several potential pathogenic mechanisms that may contribute to the decreased capacity for exercise in IR. These include metabolic and non-metabolic sequelae of IR in the vasculature and in cardiac and skeletal muscle. The literature

lends support to multiple possible mechanisms for such a relationship, including IR at the level of the vasculature leading to endothelial dysfunction (in both peripheral and cardiac circulation), IR at the level of the muscle (cardiac and skeletal) leading to a decline in mitochondrial content and/or function, and IR at the level of the heart and/or skeletal muscle leading to inefficient substrate utilization. Recent attention has been focused on changes in substrate utilization and metabolic inflexibility in IR. Simply stated, insulin promotes carbohydrate utilization. In the absence of sufficient insulin signaling in IR, the metabolism relies more heavily on fatty acids, a less oxygen-efficient fuel source. Furthermore, IR is associated with defects in mitochondrial function and oxidative capacity, which limit the ability to utilize these alternative fuel sources. These mechanisms and their potential relationship to exercise capacity are discussed briefly in the following sections.

Hyperglycemia

To date, no associations have been found between markers of glucoregulation (hemoglobin A1c or fasting serum glucose concentration) and exercise performance (23, 123, 124, 139, 140). Thus, changes in glycemic control, per se, do not appear to affect exercise performance. In light of the fact that exercise defects are associated with IR, not solely with diabetes, this result is not surprising.

Endothelial dysfunction

One possible mechanism for the exercise abnormalities observed in persons with IR invokes the associated endothelial dysfunction as a contributing factor. The exercise abnormalities observed could reflect a deficient endothelial dilator response to metabolic demand in heart as well as peripheral skeletal muscle. In this scenario, exercise capacity would be limited by peripheral and/or coronary blood flow. It is well established that peripheral endothelial function and vascular reactivity in response to pharmacological vasodilators and cuff ischemia at rest (141, 142), as well as in response to exercise, are abnormal in adults with T2DM compared to non-diabetic controls (143, 144). Furthermore, insulin's physiologic ability to enhance endothelium-dependent vasodilation is markedly impaired in diabetic individuals compared to lean control subjects, and it has been proposed that IR at the level of the endothelial cell is invariably associated with endothelial dysfunction (145). This is supported by the observation that obese subjects with and without T2DM have endothelium-dependent vasodilation that is reduced by 40 – 50% compared with lean control subjects (146). In addition, every insulin-resistant state studied to date has been found to have associated endothelial dysfunction (145). Thus, IR results in endothelial dysfunction and in impaired demand-mediated increases in muscle (and probably cardiac) blood flow, in addition to decreased

glucose transport into muscle. Prompted in part by findings in other disease states, such as heart failure, where an association between exercise performance and endothelial function has been reported (147), the relationship between endothelial function and the exercise abnormalities potentially associated with IR are being investigated further.

That endothelial dysfunction alone causes exercise defects is demonstrated by the studies of Jones et al. using *N*-nitro-L-arginine methyl ester (L-NAME) to reduce NO levels prior to performing exercise. They found a decrease in VO_2max, which correlated with the expected reduction in vasodilation and decreased perfusion of large muscle groups (148). However, in contrast to the exercise impairment in our studies with T2DM subjects, L-NAME induced an acceleration of the rate at which oxygen consumption increased with exercise (VO_2 kinetics) (148, 149). This could be explained by the observation that NO appears to competitively interfere with the mitochondrial electron transport chain (150–152), impairing muscle oxidative phosphorylation and slowing muscle VO_2 kinetics. Thus, inhibition of NO synthesis alone appears to decrease VO_2max through decreased perfusion but to speed VO_2 kinetics via the removal of NO-mediated inhibition of mitochondrial oxidative metabolism. The fact that VO_2 kinetics is slowed in diabetes implies that changes in exercise parameters in diabetes cannot be fully explained by changes in NO synthesis or, presumably, by endothelial dysfunction alone.

Myocardial dysfunction

It is likely that cardiac factors also contribute to the exercise abnormalities of T2DM and metabolic syndrome. Evidence has accumulated for the existence of myocardial dysfunction that is unrelated to coronary artery disease in many individuals with diabetes, even early uncomplicated diabetes (e.g., (153–160)). This condition has been termed “diabetic cardiomyopathy” and generally refers to a finding of subclinically impaired left ventricular (LV) function at rest (153, 156, 158, 159, 161) and/or during exercise (155, 157) in the absence of major coronary disease or hypertension. The above studies have demonstrated a predominant component of diastolic dysfunction in diabetic cardiomyopathy. Similar subclinical, largely diastolic, dysfunction has also been demonstrated in metabolic syndrome (25). Clinically it has been shown that cardiac diastolic dysfunction correlates closely with impairments in CV exercise capacity in heart failure (147), diabetes (157), and normal subjects (162). In our studies of exercise dysfunction in T2DM, we have observed that pulmonary capillary wedge pressure rises more steeply and to a greater level with exercise in T2DM than in controls (163) and that this cardiac abnormality, which may represent diastolic dysfunction, correlates with the observed decrease in exercise capacity. Thus while the prevalence, etiology, and clinical significance remain unclear, it is possible that “diabetic” cardiomyopathy plays a significant role in the exercise defects seen in T2DM and in metabolic syndrome.

Finally, we have also observed that the cardiac abnormality, which correlates with the decrease in exercise capacity in uncomplicated T2DM, also correlates with reduced myocardial perfusion (Regensteiner and Reusch, unpublished results). On the basis of these studies, impaired coronary artery endothelial function may be the mechanism for exercise impairment in T2DM via adverse effects on cardiac function. However, other data in the literature suggest the alternative or additional mechanisms discussed below.

Cardiac substrate utilization in IR

Cardiac energy production via preferential, but ineffective, use of fat over glucose could also contribute to exercise defects in diabetes. This model is supported by recent studies examining cardiac fuel utilization in IR rodents, in which a fixed, excess reliance on inefficient fat oxidation in the diabetic myocardium relative to non-diabetic controls was demonstrated (164, 165). This fuel preference occurred at the expense of glucose oxidation and was accompanied by increased myocardial oxygen consumption with less ATP produced per unit of O_2 consumed, and impaired cardiac efficiency. Similar results have been obtained in other IR animal models and in human subjects (166). For example, Peterson et al. demonstrated increased myocardial oxygen consumption, decreased cardiac efficiency, and increased cardiac free fatty acid (FFA) utilization in obese women compared to controls (167). Since ventricular relaxation is a highly energy-dependent process, ventricular stiffness and diastolic dysfunction, such as that seen in diabetic subjects, may be an early presentation of energy-poor states, including inefficient substrate oxidation.

Interestingly, increased FFA levels and utilization at the expense of glucose oxidation have also been demonstrated in ischemic myocardium in both animal models and humans (168). This fuel utilization preference has been shown to contribute to cellular acidosis and decreased cardiac efficiency in the ischemic heart and is thought to play a role in ischemic and reperfusion injury. Pharmacological stimulation of glucose oxidation with dichloroacetate, an activator of the pyruvate dehydrogenase complex, rescues these defects in rat ischemic myocardium (169). Therefore, the model of IR-induced myocardial substrate shifts may provide a mechanism not only for impaired exercise capacity but also for the worsened outcomes of acute coronary events in diabetes.

Skeletal muscle changes in diabetes

The role of skeletal muscle in the impaired exercise responses of persons with T2DM has not been specifically elucidated. However, as skeletal muscle plays an integral role in IR, it is likely that changes in skeletal muscle structure and function

may be associated with diminished exercise function. In our study of persons with T2DM in which VO_2max was lower, cardiac index was reduced by about 15% and yet arteriovenous oxygen extraction was the same compared to obese controls (163). Baldi et al. (20) also reported a reduced VO_2max, a trend towards lower cardiac output, and lower arteriovenous oxygen extraction in T2DM patients compared to controls. In their study, VO_2max correlated with the arteriovenous oxygen difference, but not with cardiac output. The findings from both groups are interesting, since even a modest reduction in cardiac output should increase reliance on oxygen extraction. The absence of an increase in the arteriovenous oxygen difference suggests that defects in oxygen transport and/or oxidative capacity of the exercising skeletal muscle exist in T2DM and may contribute to the exercise defects seen in this population.

In addition, capillary density is reduced in T2DM skeletal muscle (170), and basement membrane structures are altered (171). These structural changes could directly contribute to alterations in microvascular hemodynamics that impair O_2 exchange from capillary to myocyte, as suggested by work in diabetic rodent models (172–174). The relationship between oxygen diffusion (potentially decreased in T2DM) and exercise performance in T2DM has not been extensively explored (175, 176). However, microvascular complications of T2DM have been associated with abnormal vascular function and lowered exercise capacity (177), further supporting this mechanism as a component of the exercise dysfunction in T2DM. We have recently evaluated the T2DM VO_2 kinetics impairment in conjunction with measures of skeletal muscle oxygenation using near-infrared spectroscopy in 11 T2DM and 11 healthy, sedentary subjects (178). This combination of measurements allowed the investigation of changes in oxygen delivery relative to VO_2 at the level of the exercising muscle. In addition to slowed VO_2 kinetics, we found an altered profile of muscle deoxygenation following exercise onset in T2DM subjects, which is consistent with a transient imbalance of muscle oxygen delivery relative to muscle VO_2 in T2DM. These data suggest a subnormal microvascular blood flow increase in the skeletal muscle of T2DM subjects in response to exercise. Studies are now underway to investigate the roles of abnormal control of peripheral blood flow and muscle metabolism during exercise on the observed exercise impairment in T2DM.

There is currently debate regarding the presence of abnormalities of mitochondrial function in IR and diabetes (21, 179–181) and whether they relate to functional defects in exercise performance or simply reflect reduced content secondary to detraining (182). To date, the available data are inconclusive. Nevertheless, adults with T2DM and with IR have demonstrated reduced skeletal muscle oxidative enzyme activity (181), lower mitochondrial content (180, 183), and an increased ratio of type IIb-to-type 1 muscle fiber (184) compared to healthy subjects. Any of these factors could lead to reduced fractional oxygen extraction. Overall, it appears that the ability to deliver oxygen into the skeletal muscle as well as the ability of the muscle to utilize oxygen during exercise may be compromised in insulin-resistant states, abnormalities that may contribute to the exercise defects seen in IR.

Effects of exercise training on exercise performance in T2DM

Exercise training can substantially improve exercise performance of individuals with T2DM (15, 140, 185). Improvements in VO_2max in men and women with diabetes ranging from 8 – 30% have been documented (125, 185, 186). In addition, a decreased heart rate per submaximal workload has been reported (140), suggesting improved exercise efficiency, again similar to results in non-diabetic persons. Oxygen uptake kinetics and heart rate kinetics became faster after 4 months of exercise training in persons with T2DM, although not in non-diabetic controls, suggesting normalization of the rate of circulatory adjustment to the beginning of exercise, a process that is impaired in sedentary diabetic subjects (125). The relationship between this training effect and improved insulin sensitivity with exercise has not been explored.

Summary

The relationship between CV exercise capacity and IR is complex and involves multiple physiological systems. Furthermore, the relationship is likely to represent a cyclic causality with decreased physical activity contributing to IR and IR causing exercise impairment that may, in turn, further promote sedentary behavior. The benefits of exercise (and, conversely, the ill effects of sedentary behavior) on CV risk factors, endothelial function, insulin sensitivity, and diabetes prevention, as well as CV and all-cause mortality, are clear. Other benefits including maintenance of mitochondrial health and number and effects on hemostasis and systemic inflammation are likely, but less well defined. It also seems likely that further study will reveal other areas of benefit derived from regular exercise. On the other hand, individuals with IR who would be expected to benefit disproportionately from exercise have been shown to be relatively inactive and unfit. While the increased risk of IR in sedentary individuals is undoubtedly one contributor to this relationship, recent evidence suggests that IR may itself cause defects in CV exercise capacity. These defects, in turn, may make exercise more difficult and uncomfortable and therefore encourage sedentary behavior in the very population that would most benefit from exercise.

Clearly, the benefits of regular exercise, especially in insulin-resistant populations, are worth pursuing. Considerable thought and effort are currently going into defining the appropriate exercise prescription for insulin-resistant individuals. However, recent studies suggest that there may not be one right answer. Results from the HERITAGE study demonstrate that responses to an exercise program are heterogeneous (187–189). For instance, although mean insulin sensitivity improved, individually 58% of subjects improved, while the remaining 42% remained stable or actually became more insulin resistant. Similar results have been found for HDLC-raising and BP-lowering effects. In addition, the response in one parameter did not predict responses in other parameters. Details on timing of these measures relative to the last bout of exercise and measures of compliance with exercise were

not presented and may account for some of the variation. However, the authors argue that genetic variation accounts for nearly 50% of the heterogeneity in responses to exercise. Therefore, patient characteristics that we do not yet understand may determine the best exercise regimen.

In addition, the difficulty of establishing guidelines for exercise in insulin-resistant populations arises not only from the complexity of the system, but also from the conflict between the ideal and the realistic. In general, studies suggest that much of the benefit of exercise, especially on insulin sensitivity, arises from bout effects of exercise. Furthermore, these bout effects likely persist longer, the more glycogen-depleting the exercise. Thus, daily or near-daily exercise of at least moderate intensity and duration provides the most benefit for insulin sensitivity. However, guidelines that stress this frequency, duration, and intensity are likely to be daunting for insulin-resistant persons with impaired ability to exercise at baseline. Is it better to have optimal guidelines that no one follows or suboptimal guidelines to which patients might realistically adhere? Exercise recommendations should stress the fact that while more is better, any increase in the level of activity is likely to be beneficial. A recommendation of regular exercise/activity has the dual benefits of optimizing bout effects of exercise and of making exercise a habit, and therefore more likely to persist. It should also be stressed that exercise does not need to cause weight loss to provide very significant metabolic risk benefits. Beyond this, an individualized approach that encourages activities that are enjoyable, that works with schedule issues, and that accepts an individual's abilities, handicaps, and motivation may be the best approach. Finally, adopting an active lifestyle as an adult with a long history of sedentary behavior and possibly multiple complications of this sedentary life, notably obesity and musculoskeletal complaints, is challenging. Clearly, the optimal approach is to establish an active lifestyle and healthy behavior patterns in children and to encourage individuals to continue these patterns as adults.

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Chapter 16
Weight Loss Medications and the Metabolic Syndrome

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Keywords obesity, phentermine, orlistat, sibutramine, rimonabant, endocannabinoids, FDA, side effects, exenatide, sitagliptin, safety

Introduction

It is clear that there is a strong association between weight gain and the development of metabolic diseases, including hypertension, diabetes, and coronary artery disease, as well as increased mortality rates (1–6). It is also clear that weight loss can markedly improve insulin resistance (IR) and other markers of adverse health risks in obese individuals (7–9). The challenge is how to help obese patients achieve and maintain weight loss. Currently available treatments include diet, exercise, medications, and surgery (10). These treatment modalities go from those with limited effectiveness and low risk, to those with greater effectiveness, costs, and risk. Pharmacotherapy for obesity is a treatment approach that has intermediate effectiveness and risk between behavioral therapy and surgery and has recently been advocated as a "mainstream" treatment option that should be discussed with patients (8, 11, 12).

Despite a dramatic rise in prevalence of obesity in children, adolescents, and adults over the last 30 years (13) and the limited effectiveness of behavioral modification as a treatment approach, there remains deep skepticism among healthcare providers about the appropriateness of prescribing medications to help people manage their weight (14). This reluctance grows out of a number of firmly held beliefs. These include the idea that weight-loss medications have unacceptable side effects, have limited effectiveness, and are not paid for by most third-party payers. Some of the reluctance to prescribe medications may also come from a belief that obesity is a behavioral problem and, as a result, it is not appropriate to prescribe medications for this condition. This last idea may grow out of a deep-seated bias against obese people. These beliefs are substantial barriers to the use of pharmacological therapy for obesity in the context of metabolic disorders.

Many healthcare providers share these beliefs. The idea that weight-loss medications are dangerous grows out of a long history of unexpected serious side effects from older weight-loss medications (15). The list of these unintended consequences is

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long (16), beginning with thyroid hormone prescribed for weight loss in the late 1800s causing frank hyperthyroidism, and continuing to the use of amphetamines in the 1930s and 1940s resulting in serious problems of addiction. Aminorex was a weight-loss medication that was widely prescribed in the 1960s and 1970s that was linked to cases of serious pulmonary hypertension. As a result, it was withdrawn from the market. Very low calorie diets using gelatin as a source of protein, popular in the 1970s, were associated with a number of cardiovascular deaths. In the 1990s, following the publication of a number of studies by Weintraub demonstrating the utility of this combination, there was a dramatic rise (Fig. 16.1) in the prescription of a combination of phenterermine and fenfluramine, commonly known as phen/fen (17, 18). Unexpectedly, cardiac valvulopathy and primary pulmonary hypertension were identified as side effects of these drugs and, as a result, fenfluramine and dexfenfluramine were withdrawn from the market (19, 20). In 1998, phenylpropanolamine was removed from the market because of concerns over strokes, and in 2003, ma huang, an active ingredient in the popular over-the-counter (OTC) product Metabolife, was removed from the market because of concerns over heart attacks and strokes.

However, there is now a good deal of information about the safety of current weight-loss medications, while there is increasing recognition that many of the medications used in general clinical practice have side effects. The question, as with any prescription, is not what the risks are, but whether the benefits of the medication

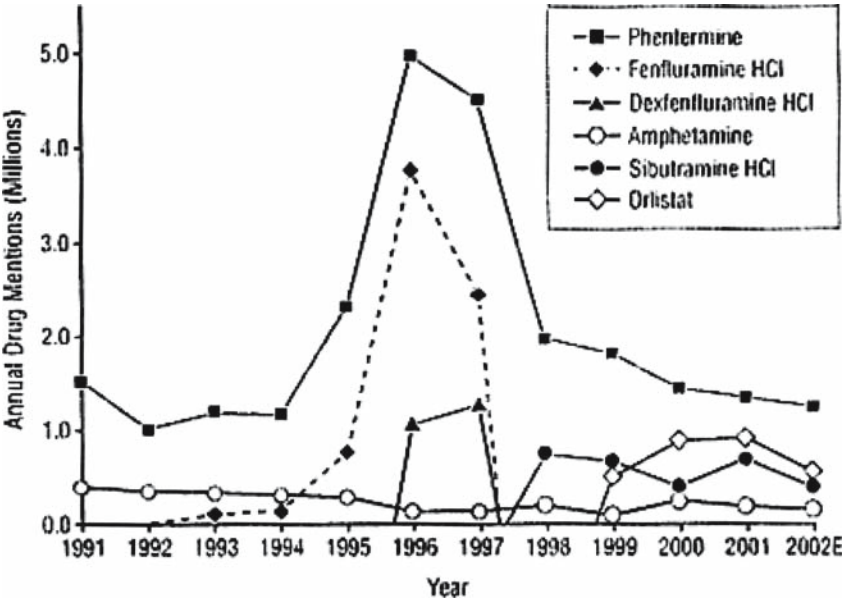

Fig. 16.1 Annual volume of anti-obesity medications reported in the United States, 1991–2002, IMS HEALTH National disease and Therapeutic Index. Data for 2002 are an estimate (E) based on January to March 2002 figures (From reference 10)

outweigh the risks. Since the history of unintended consequences associated with weight-loss medications may have created a state in which these medications are held to a uniquely rigorous standard of safety, it may be important for physicians to ask themselves whether their safety concerns over weight-loss medications are justified or are a manifestation of some other resistance to this form of treatment.

Two further concerns are that weight-loss medications are widely believed to be ineffective and are typically not covered by third-party payers. The issue of effectiveness will be discussed below, but it suffices to say these two issues are interrelated. Obesity is likely a root cause of some of the most common disorders seen in medical practice, including diabetes, hypertension, coronary artery disease, degenerative arthritis, gastroesophageal reflux, depression, breast and colon cancer, and stress urinary incontinence, to name just a few (4, 21, 22). The pharmaceutical costs attributable to these conditions are enormous, and the available data suggest that effective treatment of obesity could dramatically reduce the healthcare burden associated with them. Currently available weight-loss medications provide 5 – 8% weight loss over diet alone and this degree of weight loss is associated with improvements in markers of metabolic health. Therefore, the benefits are not trivial.

However, the number of patients needing treatment is so large that the effectiveness of current medications is limited compared to currently available medications for each of the related metabolic diseases, and the current costs are so large that insurers are understandably reluctant to take on the financial risk associated with covering weight-loss medications in the absence of clear and compelling evidence of cost benefit. Patients occasionally come to their healthcare provider with an interest in trying a weight-loss medication. In this situation, it is important to advise the patient about the likelihood that they will have to pay for the medicine and what the likely cost will be (discussed below). At that point it becomes the patient's decision as to whether the cost is justified.

Finally, it may be that the most significant barrier to physicians' prescribing weight-loss medications, and the most resistance to change, is an underlying sense that overweight and obesity represent behavioral problems that are not appropriate for treatment with medications. There is a widely held belief that if people would simply choose to eat less and exercise more, the problem could be remedied. The corollary is that if a person does not make these changes, the weight problem is really their fault (23, 24). This conviction may reflect an underlying belief that body weight is chosen rather than biologically regulated (25). However, this belief is in conflict with a substantial body of research demonstrating a strong biological component to body-weight regulation and may represent an underlying bias against obese people that is so pervasive that it is even seen among healthcare professionals who specialize in weight management (26, 27). This bias has been documented in a number of studies (28–30) and may manifest through a discomfort in interacting with obese people and a distaste for the problem and its management.

In a number of other conditions including diabetes, hypertension, and hyperlipidemia, physicians rarely blame the patient for their condition and comfortably move directly to pharmacotherapy with minimal emphasis on behavior modification, despite the fact that changes in diet and physical activity have clearly documented

benefits in these diseases. How many physicians would tell a patient with type 2 diabetes and elevated blood glucose that "we are not going to use medications in your case until you demonstrate to me that you are able to adhere to a strict diet and exercise program"? Yet, this sort of comment is commonly heard by seriously obese patients when they go to their doctor asking about a weight-loss medication.

Healthcare providers sometimes think of obesity as a "lifestyle" or "quality of life" condition that does not justify medical therapy, and yet there are clear health complications of obesity and medications are commonly prescribed for other "lifestyle" conditions. While good dietary and physical activity habits are important in the management of all of these metabolic diseases, the reluctance to use medications seems to be more strongly held when the patient is obese. Physicians often emphasize the seriousness of the side effects that a person might experience with a weight-loss medication (such as diarrhea with orlistat) and yet spend little time on potentially serious side effects from other commonly prescribed medicines (such as hypoglycemia with glyburide, hypokalemia with lasix, or myositis with statins). Unfortunately, bias is difficult to combat because it frequently goes unrecognized by the person who holds the bias. Therefore, it is important for healthcare providers who care for obese patients to consider their own opinions about obesity and how these beliefs might affect the care that is given.

Goals of Weight Loss Therapy

What is an appropriate goal for a weight-loss treatment plan? If a clinician asks his or her patient what their goal weight loss is, the answer will frequently be a 30% to 50% weight loss (31). While this would be optimal if it could be accomplished realistically, there are no treatments currently available that can provide this degree of weight loss without substantial risk. The treatment options currently available range from those of relatively low effectiveness and low risk to greater effectiveness and greater risk. Gastric bypass surgery can provide 30% weight loss but the mortality associated with this procedure is 1 – 2%, with an 8 – 20% complication rate and a lifelong change in eating behaviors (9). For most patients with mild or moderate obesity, this would seem to be an unacceptable degree of risk. On the other hand, most diet and exercise programs can provide 5 – 7% weight loss and this degree of weight loss has clearly been shown to have health benefits. In the Diabetes Prevention Program, individuals with impaired fasting glucose and/or a history of gestational diabetes were randomized to behavioral intervention, usual care, or metformin. In the behavioral weight-loss group, subjects lost 6% of their initial weight and had some regain over the subsequent four years (32). This modest degree of weight loss produced by diet and exercise was associated with a highly significant 58% reduction in the rate of developing diabetes (33). This result has been seen in other studies as well (34). These studies clearly demonstrate that 5 – 7% weight loss has meaningful effects on metabolic health. For this reason, 5 – 7% weight loss has widely come to

be viewed as medically meaningful weight loss and is the "bar" or standard against which any obesity treatment should be judged.

But is this a meaningful degree of weight loss for patients? Realistically, when a patient considers treatment for their weight they only have two choices: accept their weight as it is, which frequently means accepting gradual progressive weight gain, or attempting some form of treatment. In this context, the most important issue is whether the patient thinks that a particular degree of weight loss is superior to accepting his or her weight at its current level. While most patients do not find a 5 – 7% weight loss ideal, some may find that choice preferable to accepting their weight as it is. A number of recent studies have suggested that the combination of weight-loss medications with a good behavioral program provides more weight loss than either intervention used alone. In fact, individuals may be able to achieve a 10 – 15% weight loss with an aggressive behavioral program combined with medications (35). This is a degree of weight loss that many patients found attractive when using phen/fen. While some interpret this result to mean that weight-loss medications do not work without a good behavioral program, others believe that for optimal weight loss without surgery medications need to be added to behavioral treatment approaches.

What are the health benefits to weight loss, in particular, medication-induced weight loss? The most definitive measure for health benefits associated with weight loss would be a reduction in mortality. At this time, there are no randomized clinical trial data that demonstrate this benefit. Alternatively, weight loss could improve health as demonstrated by reduction in the incidence of serious intermediate end points, such as the development of diabetes, or through the improvement in biochemical markers of metabolic health, such as low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, insulin, glucose, and others. Weight-loss medications do demonstrate improvements in many markers of metabolic health, but the degree of improvement seen is rather modest in comparison to more traditional treatments. One advantage of weight loss as a strategy for improving metabolic health, however, is that weight loss has the potential to simultaneously improve multiple conditions, while treatment of hyperlipidemia, hypertension, or hyperglycemia with traditional medications has benefits that are more limited to the condition targeted.

Overview of Weight Loss Medications

Currently available weight loss medications provide 5%–7% weight loss over 3–6 months of use. Weight will tend to plateau at that point, with weight regain following discontinuation of the medication. This means that long-term weight-loss medications likely will need to be used to give long-term benefits (36). Some studies have been done using intermittent administration of medications (37), and this is a reasonable strategy if the patient or physician is concerned about long-term use. However, this approach does what one might expect from what is understood about the effects of these medications on body weight. Specifically, weight loss occurs

while medications are taken, stabilizes at a new lower level, begins to increase almost immediately with discontinuation of the medication, followed by restoration of weight loss with reinstitution of medication. The medications do not permanently change the way the body regulates weight, but rather their effect goes away when they are stopped. The modern view is that obesity is a chronic, typically progressive metabolic disorder, much like diabetes or hypertension. If medications are to be used to manage the condition, the medications must be used chronically.

Second, most weight-loss medications are not paid for by insurance plans and, as a result, patients will need to pay for these themselves. The cost of these medications will be listed in each section below, but ranges from US$40 to US$120 per month. This cost will be borne by the patient for the duration of therapy, which likely will be many years. Therefore, the choice of a medication for many patients depends on the mechanism of action, the cost per month, the side effect profile of the medication, and issues of the Food and Drug Administration (FDA) approval. These parameters will be highlighted with each of the existing medications. There is hope that in future there may be increased insurance coverage and, perhaps, improved efficacy with the use of combination therapy or newer medicines acting through novel pathways.

Sibutramine

Sibutramine (Meridia; Abbott) is a combination norepinephrine and serotonin re-uptake inhibitor. Its primary mechanism of action is to decrease appetite, specifically by increasing satiety. Individuals taking sibutramine may feel full earlier in a meal than they do when not taking the medicine. It is typically used at a dose of either 10–15 mg per day and there is a clear dose response both for effectiveness and side effects. Early studies using 20–30 mg/day demonstrated greater weight loss. However, these higher doses also had substantially more side effects, including a higher incidence of unacceptable rise in blood pressure. The cost of this medication is roughly US$90.00 per month.

Sibutramine has been used in more than 70 randomized, controlled clinical trials, many of 1–2 years duration, in a variety of patient types (8). Overall, sibutramine produces a 5%–7% weight loss over that seen in placebo control groups treated with behavioral modification alone (8). The Sibutramine Trial of Obesity Reduction and Maintenance (STORM) demonstrated a sustained two-year effectiveness for sibutramine (Fig. 16.2) (38). While a modest weight regain was seen in the last six months of this trial, there was a substantial weight regain in the control (placebo) arm. In multiple studies, roughly 60% of individuals taking 15 mg/day of sibutramine lost 5% of baseline weight, while a little over 30% of individuals lost more than 10% of their baseline weight. Furthermore, in the STORM trial sibutramine treatment resulted in a reduction in triglyceride levels and significant rise in HDL cholesterol at the two-year time point (Fig. 3) (38). Overall, sibutramine has been associated, in randomized controlled clinical trials, with 10%–15% reduction in triglyceride levels, 10% increase in HDL cholesterol levels, a reduction in waist circumference, and improvements in insulin sensitivity.

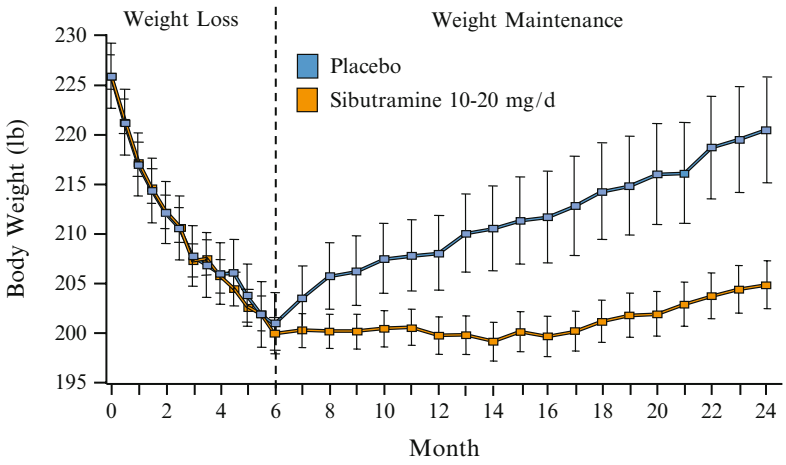

Fig. 16.2 Weight loss with sibutramine. Mean body weight changes during weight loss and weight maintenance phases. (From reference 38) (*See* Color Plate 6)

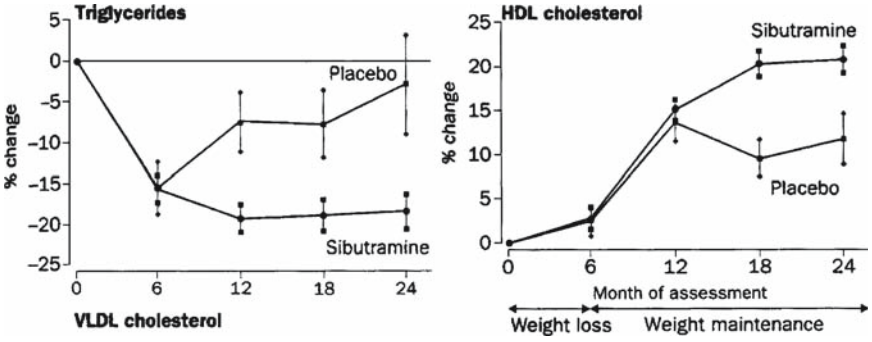

Fig. 16.3 Effects of sibutramine treatment on serum lipids (From James et al. Lancet 2000, 356:2119–2125)

Some are concerned about the continuous use of this medication. Intermittent administration of sibutramine was tested in a study by Wirth (37), the results of which demonstrated what one might expect: when sibutramine is discontinued, weight begins to rise but with reinstitution of the medication weight again falls to a level comparable to that seen in individuals using the medication continuously. Sibutramine has also been used in individuals with type 2 diabetes. In a study by Fujioka, sibutramine produced a 5% weight loss in 25% of subjects and 10% weight loss in a little more than 5% of patients (39). This study demonstrates what has been seen with other weight-loss approaches: specifically, it appears to be more difficult to produce weight loss in people with type 2 diabetes than in those without this disorder. Sibutramine has also been used in a number of studies in obese adolescents (40, 41), which have demonstrated that the drug is well tolerated and produces a significant 5%–7% weight loss that this is associated with improvements in serum lipids and a reduction in waist circumference.

The role of pharmacotherapy in conjunction with behavioral modification has also been examined with sibutramine. In a recent study by Wadden, patients were randomized to sibutramine alone, a lifestyle modification alone, sibutramine plus brief lifestyle counseling delivered by a primary care physician, or an intensive lifestyle modification program added to sibutramine treatment (40). This study demonstrated that the combination of behavioral modification plus medication works substantially better than either treatment alone (Fig. 16.4). Individuals in the combined therapy group lost roughly 12% of baseline weight. This degree of weight loss is comparable to that seen with phen/fen. While some interpret this study as demonstrating that medications do not work in the absence of lifestyle modifications, another interpretation would be that lifestyle change is less effective when delivered in the absence of medical therapy. Individuals who are

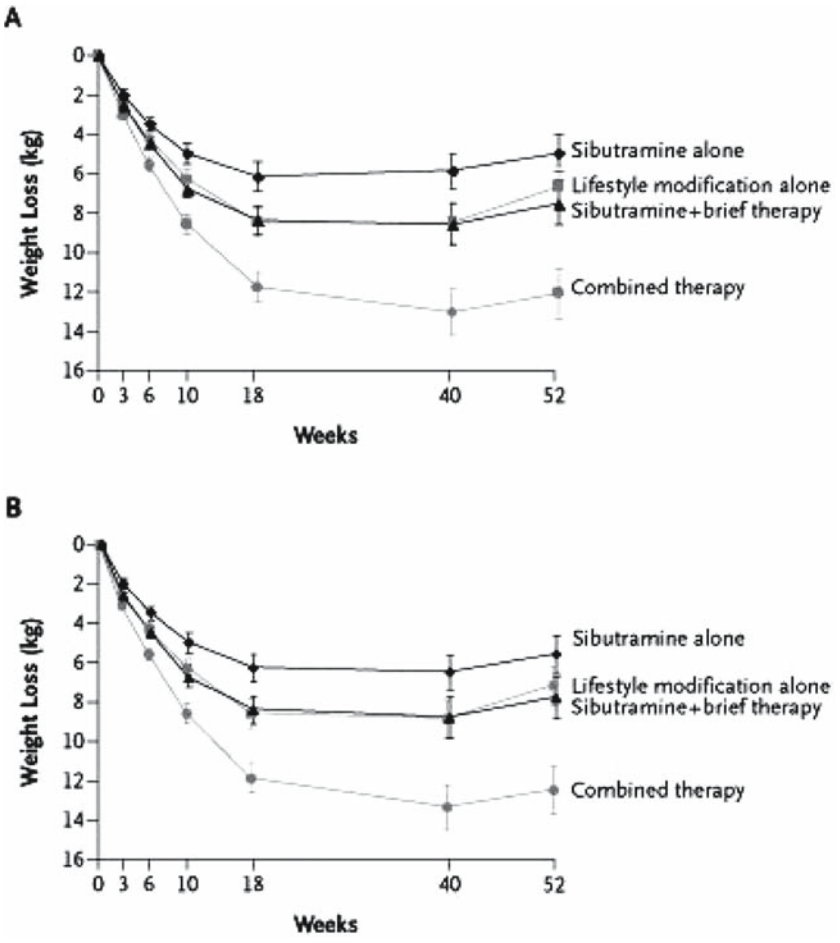

Fig. 16.4 The effects of behavioral modification, weight loss medications alone, or in combination, on weight loss. Mean (± SE) weight loss in the four groups, as determined by intention-to-treat analysis (Panel A) and a last-observed-carried-forward analysis (Panel B) (From reference 35)

most successful in this group were the ones who adhered most closely to the recommendations of self-monitoring of diet.

Sibutramine has a number of adverse affects, including dry mouth, constipation, insomnia, and dizziness. The most concerning potential side effect is hypertension. In roughly 1% of subjects, blood pressure rises to a point where the medication needs to be discontinued. Tachycardia is also seen with a modest rise in pulse (3–5 bpm) in many patients. It is important to start sibutramine at a dose of 10 mg/day and to monitor the patient's blood pressure for 1–2 weeks. If a person tolerates the medication well for the first month, the dose can be increased to 15 mg/day and continued at that level.

Orlistat

Orlistat (Xenical; Roche) is a pancreatic lipase inhibitor that works by blocking fat absorption by roughly 30%. The medication is given as 120 mg by mouth with each meal and the cost is roughly US$120.00 per month. Orlistat has been studied in over 30,000 patients for up to four years in over 90 controlled clinical trials in a range of patient types (8, 42). Like sibutramine, orlistat can deliver a 5% to 7% weight loss above that seen with a behavioral treatment program alone. The effectiveness of orlistat therapy given for two years was demonstrated in a study by Sjostrom (Fig. 16.5) (43). As has been seen with other weight loss medications, the benefits of orlistat go away on discontinuation. In a number of studies, the fraction of patients achieving a 5% weight loss is between 60% and 70% on orlistat therapy, while 30% of patients achieve greater than 10% weight loss. As has been seen with sibutramine, orlistat has a number of metabolic benefits in addition to simply producing weight loss. These include reduction in LDL cholesterol levels, serum triglyceride levels (44), and waist circumference, and improvements in blood glucose and HbA1c in people with type 2 diabetes (Fig. 16.6) (39, 45). The magnitude of these metabolic benefits is similar to those seen with either sibutramine or rimonabant (discussed below).

Orlistat has also been tested for the prevention of the development of type 2 diabetes in at-risk individuals (46). In the Xenical in the prevention of Diabetes in Obese Subjects trial (XENDOS), high-risk individuals were randomized to either placebo or orlistat. Fifty-three percent of individuals randomized to orlistat achieved a 5% weight loss or greater, and 26% achieved a 10% or greater weight loss at four years of follow-up. The four-year incidence of type 2 diabetes in the placebo group, already receiving diet and exercise counseling, was 9%. The four-year incidence of type 2 diabetes in the orlistat group was 6.2%, representing a 37% reduction in the incidence of diabetes, which was highly significant. The use of orlistat as a weight-loss agent has also been examined in obese adolescents in several studies (47–49). These studies demonstrated an effectiveness that was almost identical to that seen in adults with similar side effects. The weight loss produced in adolescent subjects was associated with improvements in waist circumference and serum lipids, as well. While there remains a good deal of reluctance to use

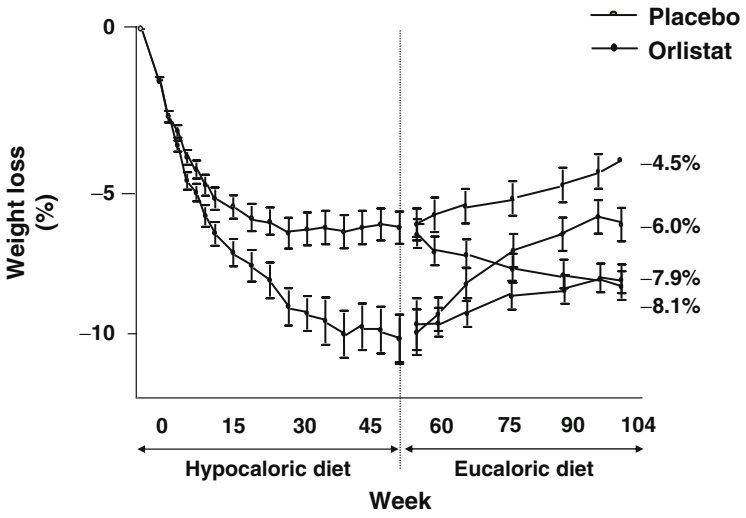

Fig. 16.5 The effect of orlistat therapy on body weight. Mean percentage change in bodyweight from start of single-blind lead-in until 2-year examination in orlistat and placebo groups. Error bars = SE (From reference 43)

medications to treat obesity in young people, these studies demonstrate the effectiveness and safety of orlistat in this population.

The side effects of orlistat relate to its mechanism of action in blocking dietary fat absorption. Patients may experience oily stools, increased frequency of bowel movements, and some sense of fecal urgency. However, if patients are made aware of the drug's mechanism of action and are encouraged to consume a reduced-fat diet, these side effects can be managed in most patients and do not often result in discontinuation of therapy. There have been concerns about orlistat producing deficiencies in fat-soluble vitamins. However, the incidence of this side effect in clinical trials has been less than 5%. It is still advisable to encourage patients to take one multiple vitamin per day to prevent the development of vitamin deficiencies on orlistat therapy. There are concerns that orlistat could alter the prothrombin time in patients treated with the oral anticoagulant coumadin, and that it also could reduce plasma levels of cyclosporine in patients receiving this medication following an organ transplant. This medication should be taken with special care and monitoring, or avoided in patients taking these medications.

Because of the demonstrated long-term safety and effectiveness of orlistat, the FDA has recently approved a 60 mg dose of this medication for OTC sales. It is the only OTC weight-loss medication that is approved by the FDA. It is being marketed by GlaxoSmithKline under the name Alli. A 60 mg dose will cost between 60 cents and US$1 per tablet. Studies presented to the FDA demonstrate that this dose taken three times per day with meals produces a 2 –3% weight loss over that seen with diet control alone. It may be that this medication will be targeted towards overweight patients, since data suggest that this lower dose produces less weight loss in these overweight individuals, as compared to the higher dose being used in obese patients.

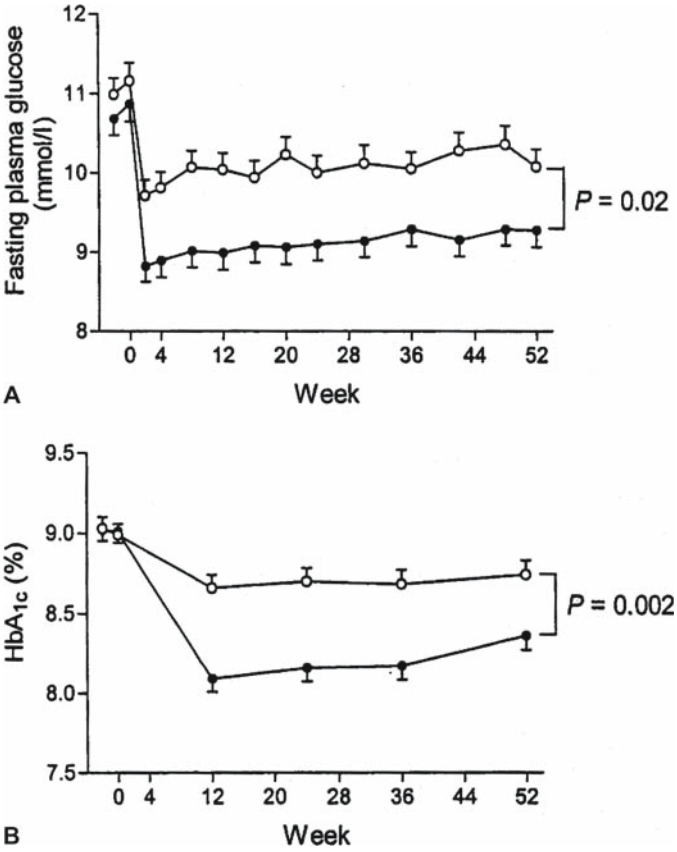

Fig. 16.6 The effects of orlistat treatment on glucose and HbA1c in patients with type 2 diabetes. A: Fasting serum glucose levels over time with placebo or 120 mg orlistat. $P = 0.02$, least-squares mean differences from placebo in the change from baseline over 52 weeks. B: HbA1c over 1 year of double-blind treatment with placebo or 120 mg orlistat. $P = 0.002$, least-squares mean difference from placebo in the change from baseline over 52 weeks. Open circles = placebo, black circles = orlistat 120 mg (From reference 45)

Phentermine

Phentermine is an older medication that increases the norepinephrine content in the brain and was half of the combination therapy, phen/fen. It produces a 5 –7% weight loss over and above treatment with diet alone. It is chemically related to amphetamine but does not have the same addictive potential. Although it is available as a generic medication, it is also marketed under a number of brand names including Obytrim, Ionamin, Adipex P, Fastin, and others. It is available as 15, 30 or 37.5 mg tablets to be taken once per day and costs US$35–45 per month. Probably because of the low cost, phentermine is the most widely prescribed anti-obesity drug in the United States at this time (18). There is currently no evidence of any serious

side effects when used as a single drug. Specifically, there is no evidence that it causes either primary pulmonary hypertension or cardiac valvulopathy.

However, in the original studies from the 1960s this medication was used only for three months, and as a result is FDA-approved only for three months of use. Therefore, many physicians are reluctant to prescribe it. As mentioned above, our current understanding of medical therapy for obesity suggests that if a medication is used only for a limited time, it will not produce a lasting change in weight. This leaves physicians in a position of having to decide to either not prescribe this medication or to prescribe it "off label". While some states have given clear regulatory guidance that it is inappropriate to prescribe phentermine off label, many states have not established clear guidelines, leaving the decision to the discretion of the patient and the physician.

The lack of FDA approval for long-term use of phentermine raises the question of whether it is ethical to prescribe phentermine off label. There are three ethical principles that could be considered in this situation. The first is beneficience. This is the professional obligation to do what the physician can to help the patient. Phentermine is the only weight-loss medication that some patients on a limited budget can afford. In addition, it clearly can produce 5 – 7% weight loss in many patients, a degree of weight loss that would be expected to have some metabolic and health benefits. The second principle is non-maleficience. This is the principle of doing no harm. Phentermine does have side effects including the potential to raise blood pressure, produce tachycardia, nervousness, some difficulty falling asleep, headaches, and potentially emotional effects such as anxiety. However, other medications that are prescribed also have side effects, which need to be weighed against the benefits before a decision can be reached about prescribing. The final ethical principle is autonomy. This is the principle that patients have a right to make an independent decision about their health. It also means that the prescribing physician has an obligation to make an independent decision whether to prescribe. Each has "veto power".

These decisions need to be based on a careful weighing of the risks and benefits for an individual patient and involve a process of informed consent. The purpose of informed consent is not to insulate the provider from legal or professional responsibility but, rather, to ensure that both the patient and the provider have carefully thought through the risks and benefits involved in the use of any medication. It is important, if a provider chooses to prescribe phentermine, that they begin at a low dose, 15 mg/day, and follow blood pressure and pulse over the next several weeks. If the patient is tolerating the medication well, the dose can be increased to 30 mg/day in 1–2 months, and again the blood pressure should be monitored. The medical record should clearly reflect a careful discussion of risks and benefits and a discussion on the lack of FDA approval for long-term use.

Rimonabant

Research directed at understanding the central pathways mediating the effects of the plant *Cannabis sativa* (marijuana) uncovered a novel endogenous cannabis-like neurotransmitter system: the endocannabinoid system. This system appears to be an important regulator of many physiological functions, including energy balance, lipid

and glucose metabolism, and food consumption. Two receptor subtypes have been identified mediating endocannabinoid action: cannabinoid-1 and 2 (CB-1and CB-2). CB-2 receptors are predominately found in the immune system. CB-1 receptors dominate in the physiological regulation of energy balance and are located throughout the body. CB-1 receptors are found both in the brain and in several peripheral sites, including the gastrointestinal tract, adipose tissue, pituitary gland, liver, and muscle. Stimulation of CB-1 receptors results in the promotion of *de novo* lipogenesis and triglyceride accumulation in the liver, reduced glucose utilization by muscle, orexigenic effects in the brain, and a reduction in adiponectin secretion by adipose tissue (50). Compared to lean controls, subjects with type 2 diabetes have greater serum levels of endocannabinoids (51). Rimonabant is the first of a class of orally administered CB-1 blockers to be studied in human subjects.

Four studies with over 7000 subjects have been published evaluating the efficacy of rimonabant in overweight or obese individuals, individuals with hyperlipidemia, and obese subjects with type 2 diabetes. These trials go by the shortened name of "RIO" for rimonabant in obesity. The first two studies published, the "RIO-North America" and "RIO-Europe", included subjects with a body mass index (BMI) ≥27 kg/m^2 with hypertension or dyslipidemia, or BMI ≥30 kg/m^2. Subjects in these studies were treated for two years with 5 or 20 mg/day rimonabant or placebo in addition to a hypocaloric diet (600 kcal/day reduction in energy intake) and instruction in exercise (52, 53). "RIO-Lipids" randomized subjects with a BMI 27–40 kg/m^2 and untreated dyslipidemia to 5 or 20 mg of rimonabant or placebo in addition to a hypocaloric diet (54). "RIO-Diabetes" included subjects with type 2 diabetes treated with metformin or a sulfonylurea, who had a hemoglobin A1c between 6.5 and 10% (55).

The primary end points for all four studies were the change in weight produced by drug treatment and the number of subjects able to achieve ≥5% and ≥10% weight loss. Secondary end points in RIO-North America, Europe and Diabetes included changes in waist circumference, glucose, insulin, cholesterol, and the frequency of metabolic syndrome within the cohorts. A weight loss of 6.6–6.9 kg was achieved in one year on rimonabant 20 mg/day, compared to 3.1–3.4 kg with 5 mg/day rimonabant and 1.5–1.8 kg with placebo in these studies (Fig. 16.7). Using an intention-to-treat analysis, 50.9% of subjects lost ≥5%, and 27.4% lost ≥10% of their body weight. This is a degree of weight loss that is comparable to that seen with the three currently available weight-loss medications discussed above. Weight loss appeared to be maintained for the duration of the trials. A significant reduction in waist circumference of 6.5–7.1cm was seen in the 20 mg/day rimonabant groups vs. 3.5–3.9 cm in the 5 mg/day group and 0.3–2.4 cm with placebo. Increases in HDL cholesterol levels of 10 – 15%, decreases in TG levels (Fig. 16.8), and improvements in measures of insulin resistance were seen in all studies and were significant in the rimonabant 20 mg/day groups. There was no change in secondary end points with rimonabant 5 mg/day except in HDL concentrations. Finally, there was a 53.6% reduction in the metabolic syndrome criteria as defined by the National Cholesterol Education Programs' Adult Treatment Program III criteria (NCEP-ATP III) with rimonabant 20 mg. (56, 57). In RIO-Lipids, a significant increase in adiponectin levels and a significant decrease in leptin levels were seen in the 20

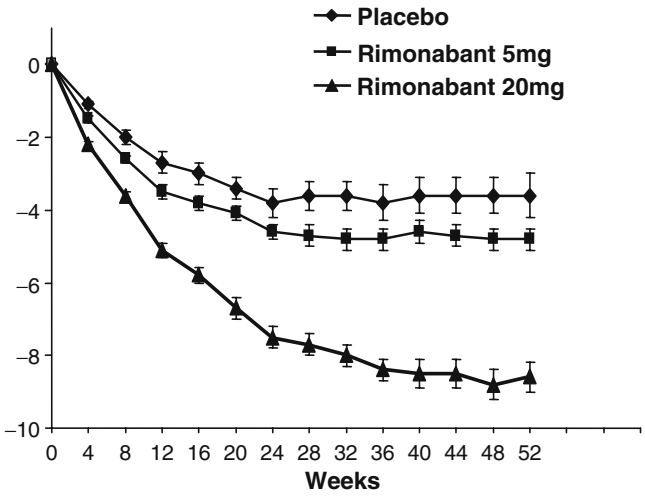

Fig. 16.7 The effects of rimonabant treatment on body weight: RIO-Europe. Data are mean (SE) values for patients completing each scheduled visit, and LOCF (values for the full ITT population with the last observations carried forward). *$p < 0{\cdot}001$ vs. placebo. †$p = 0{\cdot}002$ placebo. (From reference 52)

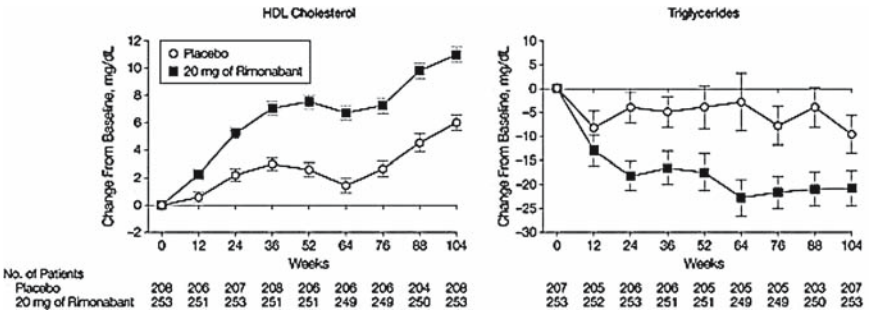

Fig. 16.8 The effects of rimonabant treatment on serum lipids: RIO-North America. Change in the completer's population from baseline over years 1 and 2 for levels of high-density lipoprotein (HDL) cholesterol and triglycerides. Errors bars indicate SEM. (From reference 53)

mg/day rimonabant group (54). RIO-Diabetes confirmed the weight-loss benefits seen in the three previous trials in patients with diabetes. Waist circumference and serum triglycerides decreased, and HDL cholesterol levels rose. Subjects taking 20 mg/day rimonabant demonstrated a modest improvement in HbA1c levels of 0.6%, with a greater number of patients achieving HbA1c of < 6.5 and < 7% compared to placebo or rimonabant 5 mg (58). While statistically significant, this effect on glycemia is modest in comparison to other available treatments for diabetes, although it was an effect that was additive to that seen with metformin or a sulfonylurea.

RIO-North America also looked at the prevention of weight regain after the first year. Subjects were initially randomized to placebo vs. rimonabant for the first year,

and a subset of subjects who received rimonabant during the first year were randomized in the second year to either placebo or continued treatment with rimonabant. Those subjects initially randomized to placebo remained on placebo throughout the two-year study. Weight loss at two years was maintained on 20 mg of rimonabant, while weight re-accumulation above that seen in the placebo-alone group occurred with subjects re-randomized to placebo following one year of 20 mg rimonabant (59).

Adverse side effects were seen in some subjects on higher doses of rimonabant. These included nausea, dizziness, and diarrhea. Because of the neuromodulatory effects of rimonabant, special attention must be paid to psychiatric adverse events. A number of subjects experience depression, anxiety, and agitation while taking rimonabant 20 mg/day compared to those on placebo or 5 mg /day rimonabant. For example, in the RIO-Diabetes study, there was a 3% rate of depression in the 20 mg/day group as compared to 0.9% in the placebo group. Anxiety was present in 0.6% of the high-dose rimonabant group, as compared to 0% in the placebo group. Overall, there was a relatively high drop-out rate of > 40% seen in each of these trials, but this has been seen in previous studies of sibutramine and orlistat and likely represents dissatisfaction with the modest weight loss produced by these medications vis-à-vis serious side effects.

Looking at these four studies together, it appears that rimonabant offers a new class of medications to the available options for overweight and obese individuals who have not had adequate response to diet and exercise treatment. However, the degree of weight loss and magnitude of health benefits of this new medication do not appear to be substantially greater than what has been seen with existing medications. Furthermore, the side effect profile has raised concerns and, to date, the FDA has not approved rimonabant for marketing.

Medications that promote weight gain

When seeing an overweight or obese patient, it is important to consider whether medications are contributing to weight gain (60). A number of antipsychotic agents (61), progestational agents, glucocorticoids, antidiabetic medications (sulfonylureas, thizolidinediones, insulin), and other medications can promote weight gain. If a patient is on one of these medicines, it is important to monitor their weight and, if weight is rising dramatically, consider other options. The other options include using a different medication, reducing the dose, or deciding whether the risks of continued administration outweigh the benefits of that medication.

There are a number of alternative medications that have some utility in this situation. Bupropion (Wellbutrin; GalaxoSmithKlein) is an antidepressant that not only has less weight gain than other antidepressants, but it actually may produce gradual weight loss (62). This is a dose related effect, with a dose of 400 mg/day producing more weight loss than 300 mg/day. If a patient is experiencing weight gain on another antidepressant, it may be appropriate to consider a switch to bupropion. Topiramate (Topamax; Ortho-McNeil Neurologics) and zonisamide (Zonegran; Eisai Inc.) are

two antiseizure medications that have also been used for headaches and as mood stabilizers. These medications have a number of neurological side effects but can produce moderate weight loss in many individuals. A number of studies examined whether topiramate could be used as a weight-loss medicine (63, 64). However, while showing moderate weight loss, these studies raised concerns about side effects, and as a result the company has not pursued an FDA indication for weight loss. While neither of these medications is FDA-approved for weight loss, if a patient has problems with migraines or a seizure disorder and is obese, these medications may have an added benefit of helping them manage their weight.

Criteria for Weight Loss Medications

The FDA provides guidance to pharmaceutical manufactures about criteria for gaining approval for a new anti-obesity drug. The current guidance was drafted in 1996 and states that drug companies must provide a dose-ranging study with at least three doses of the medication. They must provide a pivotal trial with at least 1500 subjects completing one year of observation and 200 to 500 subjects completing two years of follow-up with the new medication. The FDA requires a six-week run-in period with behavioral modification, and the studies should include relevant end points, such as blood lipids, glucose, and insulin. The level of effectiveness required by the FDA is a 5% placebo-subtracted weight loss for a new agent. These guidelines are currently under review, and in February 2007, new draft guidelines were released for public comment (www.fda.gov/cder/guidance/7544dft.pdf). These draft guidelines confirm many central elements of the previous guidelines, including the definition of patients with a BMI > 30 kg/m^2 or > 27 kg/m^2 with co-morbidities as appropriate for consideration of treatment with weight-loss medications, the importance of measuring effects on lipids and glucose metabolism, and the efficacy benchmark of 5% placebo-subtracted weight loss. The new guidelines suggest 3000 subjects be treated with the drug for at least one year in a pivotal trial. Important new components include guidance on the use of weight-loss medications in children and adolescents and in patients with drug-induced weight gain, and the use of combination therapy for weight loss.

With the dramatic growth in our understanding of the systems involved in body weight regulation, a large number of new targets for anti-obesity drugs have been identified. Because of the potentially large market for an effective anti-obesity medication, a growing number of small and large drug companies now have anti-obesity compounds under investigation. In the United States, there are roughly 65 million people with high blood pressure. In 2004, almost 43 million of these individuals were being treated chronically with anti-hypertensive medications. The situation for drug treatment of obesity is quite different. There are roughly 65 million obese Americans, yet only 2.5 million of those have ever received a prescription for a weight-loss medication, and the average duration of therapy for an anti-obesity

drug is only 90 days per year compared to 200 days per year for anti-hypertensive medications. This potential market, along with a large number of potential drug targets, has fueled a dramatic rise in investment in potential compounds. Hopefully, this investment will bear fruit in the coming years. In the next section, a number of newer medications that have weight-loss effects, and recent experience with combination therapy will be discussed.

Newer Anti-diabetic Medications with Weight Loss Effects and Newer Approaches to Combination Therapy

In the last few years, three new anti-diabetic medications that have effects on weight have entered clinical use. These include pramalintide, exenatide, and sitagliptin. Pramalintide (Symlin; Amylin Pharmaceuticals) is a synthetic analog of the β-cell hormone amylin (65). It is a parenterally administered compound that has been FDA-approved since 2005 as an adjunctive anti-diabetic medication for patients with both type 1 and type 2 diabetes. Exenatide (Byetta; Amylin pharmaceuticals and Eli Lilly & Co.) is an incretin mimetic approved as adjunctive therapy for people with type 2 diabetes who are using metformin, a sulfonylurea, or a thiazolidinedione (TZD) alone or in combination without achieving adequate glycemic control. Sitagliptin (Januvia; Merck & Co) is an oral, selective dipeptidyl peptidase-IV (DPP-4) inhibitor developed for the treatment of type 2 diabetes (66). It produces a 90% reduction in the activity of the enzyme that is responsible for the metabolism of glucagon-like peptide-1(GLP-1). The effect is to increase circulating levels of GLP-1. The advantage of medications that work on this pathway is that they can be administered orally avoiding the injection that is required with GLP-1-like medications.

Improving average levels of glycemia is a primary goal in people with type 2 diabetes. Unfortunately, weight gain is a common side effect of many of the medications used to accomplish this goal. In addition, while obesity is common in people with type 2 diabetes, as was noted above, it may be more difficult to produce and maintain weight loss in these individuals. The availability of these new medications to control glucose without producing weight gain is therefore particularly relevant. While there have been a limited number of studies to date on the weight effects of these medications, it appears that pramlintide produces a greater degree of weight loss than the other two newer anti-diabetic medications, a degree of weight loss that is comparable to other available weight-loss medications (67–69). In one study, 400 obese subjects were randomized to a range of doses given parenterally, and pramalintide produced a 4 – 6% weight loss over a 12-week period. Exenatide is somewhat less effective, with a 2 – 4% weight loss in people using 10 μg of exenatide twice a day (70, 71). Much less is known about the weight effects of sitagliptin. While it has the advantage of being orally administered and appears to have beneficial effects of glycemia in people with type 2 diabetes, so far

it looks like the weight-loss effects are modest, around 2 – 3% (72, 73). None of these medications has an FDA indication for weight loss *per se* and should not be prescribed for this indication.

It seems likely that anti-obesity medications will not be widely prescribed and used until they produce a degree of weight loss that patients and physicians believe to be clinically significant. Experience with phen/fen and existing agents suggests that this threshold is greater than 5%, and may be close to 12 – 15% weight loss. It seems logical that a combination of medications working by different mechanisms would have greater effectiveness then single agents, as has been the case in other disease states. Serious high blood pressure cannot be effectively treated with a single agent, and yet the combination of a potent vasodilator, a diuretic, and a beta-blocker provides excellent blood pressure control even in those with severe hypertension. Combination therapy has also been invaluable in managing severe hyperlipidemia, infections, and other health problems. In an effort to achieve a higher level of effectiveness in weight loss, combination therapy is being extensively evaluated by number of companies at this time.

Qnexa (Vivus Inc.) is a patented combination of Topiramate and phentermine that has been used in a clinical trial of 200 overweight subjects. These individuals lost roughly 12% of their body weight, compared to 2% in the control group over the initial 12-week trial. This is the kind of effectiveness that may prove to be desirable to both providers and patients. Orexigen therapeutics Inc. a company devoted to the development of combination therapy for obesity, is currently exploring two combinations. Contrave is a bupropion/naltrexone combination that has been used in an early 24-week trial and found to produce a 7% weight loss. Excalia is a combination of bupropion and zonisamide that was used in a 48-week trial and found to produce a 9 – 12% weight loss. Contrave is in phase III clinical trials now, Excalia in late phase II. Amylin has been combined in animal studies with PYY and leptin to produce 16 – 18% weight loss. The company is now beginning studies in humans using this combination therapy. These early unpublished experiences provide early evidence as to the utility of combination therapy in the treatment of obesity.

Summary

Obesity is a serious and growing problem in the United States and around the world. It is clearly associated with an increased risk of developing a wide range of metabolic disorders and a reduction in both the length and quality of life. While behavioral treatment approaches are the cornerstone of management, pharmacotherapy offers added efficacy without a risk of serious side effects. It is important for physicians to have a good working knowledge of how and when to consider prescribing weight-loss medications. It is hoped that over the coming years newer weight-loss medications used as single agents or in combination will provide obese patients with the kind of weight loss that will truly improve their health and quality of life.

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Color Plates

Color Plate 1

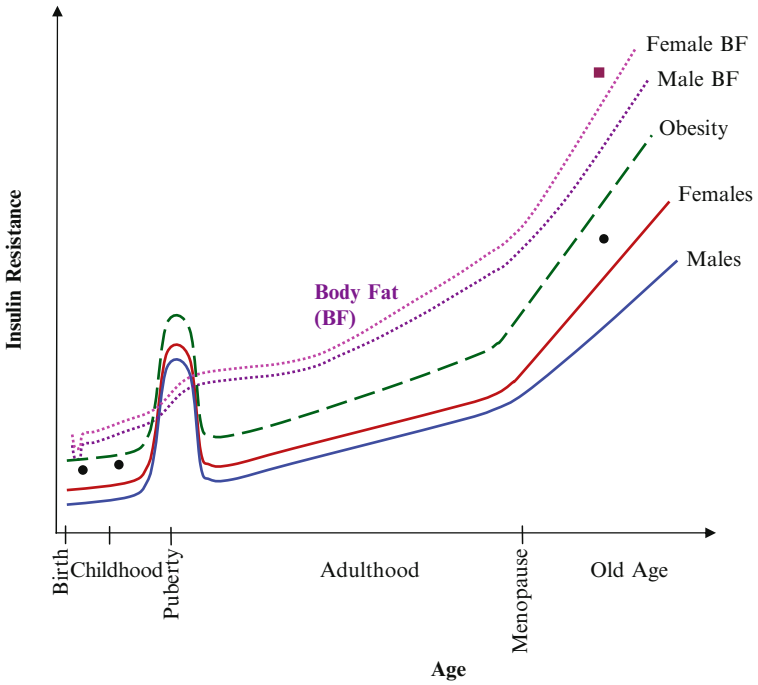

Fig. 8.1 Representation of insulin resistance and body fatness (BF) throughout life. Over all stages of the life cycle, from birth to old age, insulin resistance (solid lines) and adiposity (dotted lines) exhibit parallel trends. The only exception is puberty, a time of profound insulin resistance, which does not directly correlate with the adiposity trajectory. However, even during puberty, those adolescents with higher body fat (i.e. obese) are more insulin-resistant than their lean peers and are more likely to develop the metabolic syndrome as adults. Females are slightly more insulin-resistant and have a higher body fat percentage than males from early childhood through old age. The black dots represent the insulin resistance of low-birth-weight infants at various ages, which is always higher than their normal-birth-weight counterparts. The plum square indicates the body fatness of low-birth-weight infants at old age. This higher percent body fat is associated with the increased insulin resistance represented by the corresponding black dot (*See* Page 136)

Color Plate 2

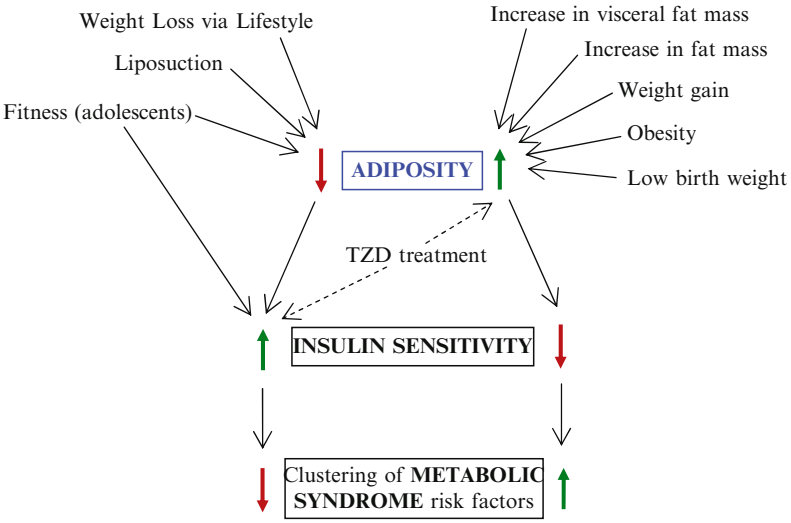

Fig. 8.2 Representation of the factors influencing whole body adiposity and, hence, insulin sensitivity. Factors that increase adiposity act to decrease insulin sensitivity and are associated with the development of metabolic syndrome in adulthood. The associations shown in this diagram apply through all stages of life – from childhood through adolescence and into adulthood. The only exception to this association between increasing adiposity and decreasing insulin sensitivity occurs in subjects treated with thiazolidinediones (TZDs), in whom whole-body fatness increases but so too does insulin sensitivity. This effect, however, is mediated through the TZD's ability to redistribute body fat to peripheral subcutaneous depots and away from visceral fat. In addition, physical fitness in adolescents may influence insulin sensitivity both via decreased total body adiposity and via a direct, fat-independent mechanism (*See* Page 137)

Color Plate 3

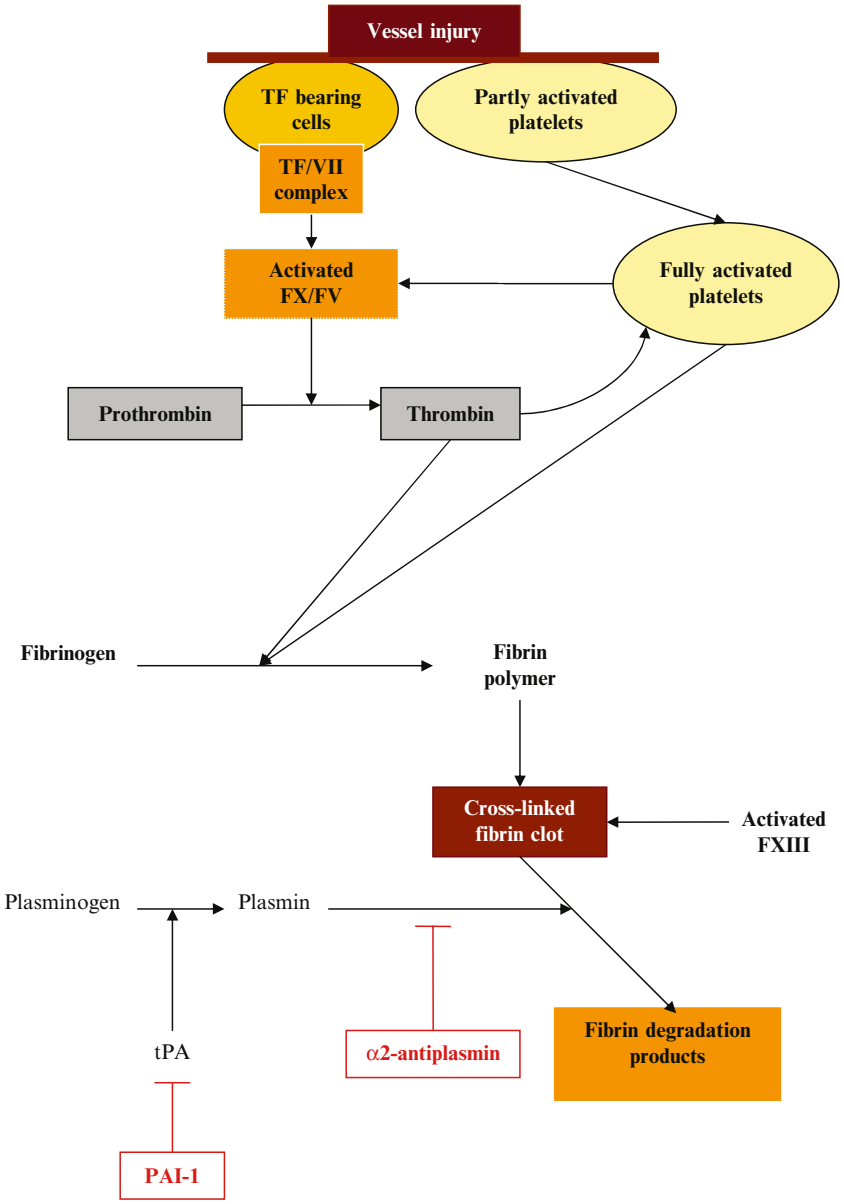

Fig. 11.1 The coagulation cascade in atherothrombotic disease. A break in the vessel wall brings the plasma into contact with tissue factor (TF) bearing cells, and platelets adhere to the site of injury and become partially activated. FVII/TF complex activates other clotting factors, which subsequently cleave prothrombin to generate thrombin, which fully activates the attached platelets, and this helps to generate enough thrombin to maintain clot formation, consequently resulting in the formation of a stable hemostatic plug.Fibrin clot lysis is initiated by tissue plasminogen activator (tPA) that mediates the activation of plasminogen to plasmin. Inhibitors of fibirnolysis include plasminogen activator inhibitor (PAI-1), which inhibits tPA, and α2-antiplasmin, which inactivates plasmin (*See* Page 188)

Color Plate 4

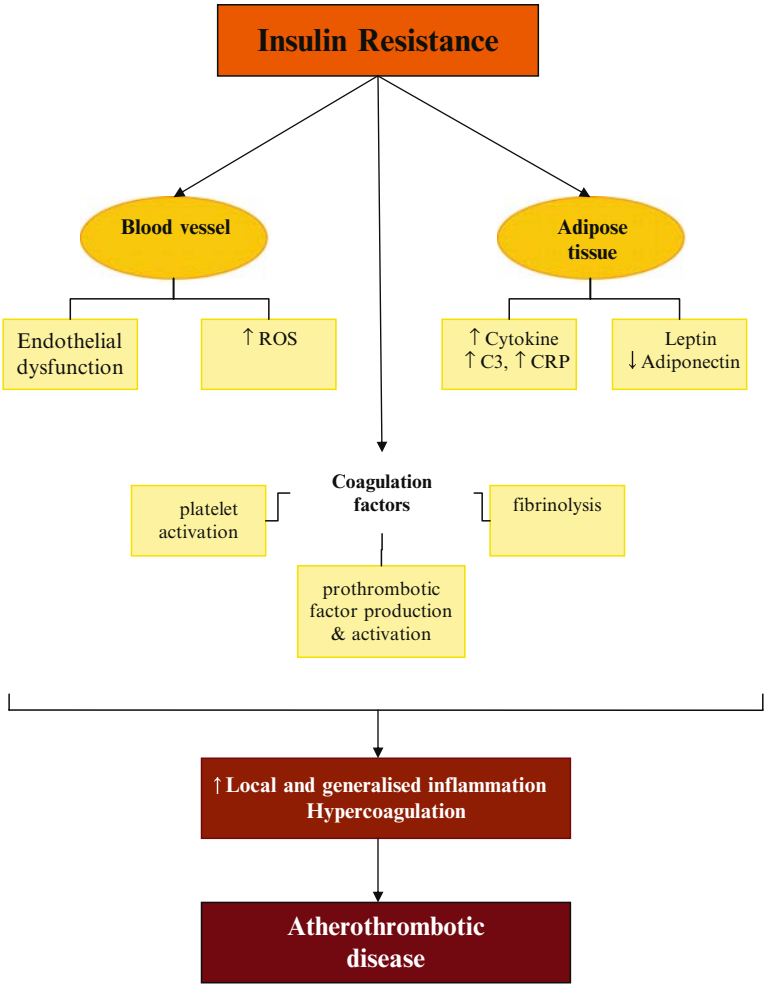

Fig. 11.2 The role of insulin resistance in the atherothrombotic process. Insulin resistance is associated with endothelial dysfunction and the accumulation of reactive oxygen species (ROS) in the vessel wall that help to start and maintain the atherosclerotic process. Insulin resistance affects the adipocyte function, resulting in the generation of a number of inflammatory molecules, which play a key role in the atherosclerotic process. Furthermore, insulin resistance is associated with increased platelet activation as well as increased level and activity of prothrombotic factors, thereby ensuring the presence of a prothrombotic milieu. CRP: C-reactive protein; C3: complement C3 (*See* Page 191)

Color Plate 5

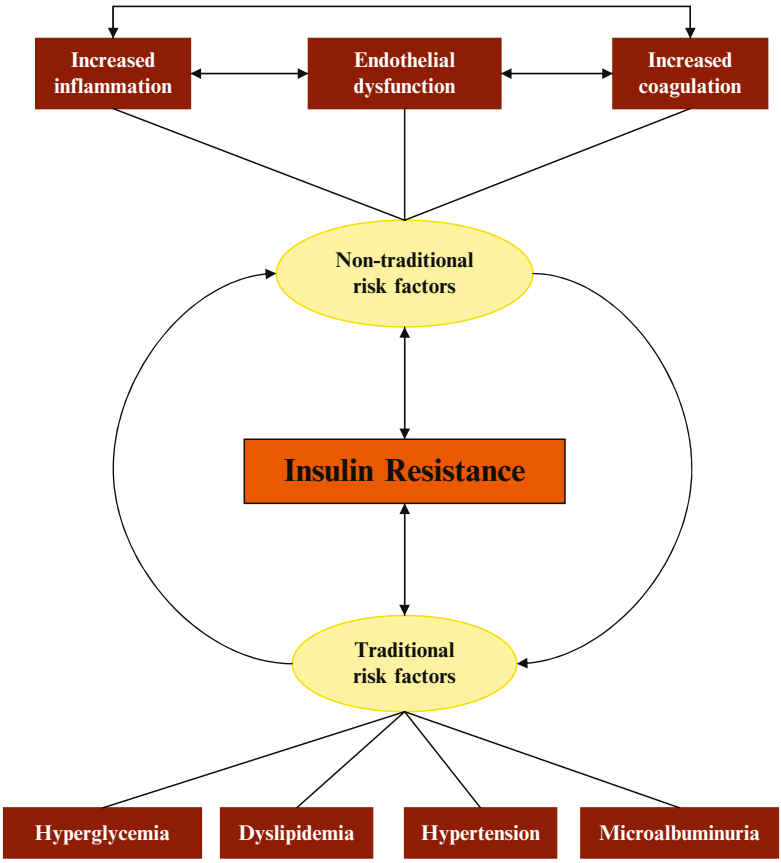

Fig. 11.3 The relationship between insulin resistance and traditional/non-traditional risk factors for atherothrombotic disease. Insulin resistance is associated with traditional risk factors for atherothrombotic disease including hyperglycemia, dyslipidemia, hypertension, and microalbuminuria. Insulin resistance is also associated with non-traditional cardiovascular risk factors including endothelial dysfunction, increased inflammation, and hypercoagulation. Furthermore, there is an interaction between traditional and non-traditional risk factors, and both can worsen insulin resistance, consequently leading to a vicious cycle that promotes the development of atherothrombotic disease (*See* Page 192)

Color Plate 6

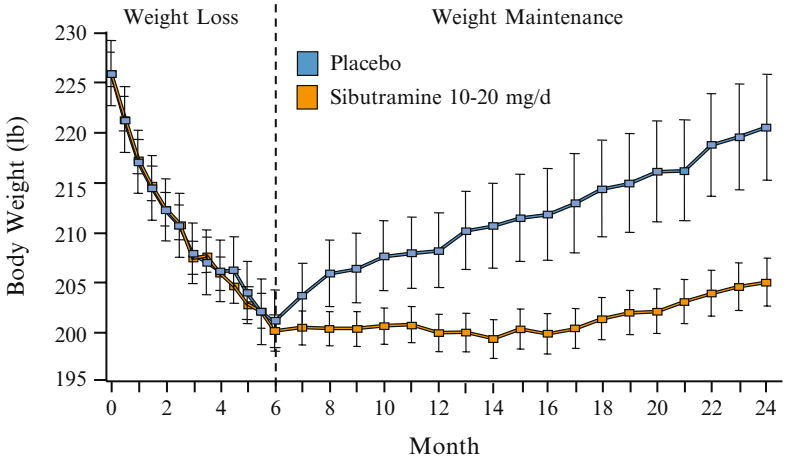

Fig. 16.2 Weight loss with sibutramine. Mean body weight changes during weight loss and weight maintenance phases. (From reference 38) (*See* Page 307)